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STUDIES ON SPATHIDIUM SPATHULA

I. THE STRUCTURE AND BEHAVIOR OF SPATHIDIUM, WITH SPECIAL REFERENCE TO THE CAPTURE AND INGESTION OF ITS PREY

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EIGHT TEXT FIGURES AND ONE PLATE (FIGURES NINE TO TWENTY)

The holotrichous infusorian which today bears the name Spathidium spathula apparently was first described by Müller in 1773, and again in 1786 as "Enchelis cylindrica, apice hyalina spathulata" and designated Enchelis spathula.[1] The next observations of importance were those of Ehrenberg[2] in 1830 and 1838, which led him to place the organism in the genus Leucophrys as L. spathula. In 1841 Dujardin[3] founded the genus Spathidium for an organism which he considered identical with Müller's Enchelis spathula, and named it Spathidium hyalinum. Eighteen years later, in his monograph on the Infusoria, Stein[4] merely mentioned the organism incidentally, and apparently was of the opinion that it was not specifically distinct from Ehrenberg's Enchelys farcimen, and retained it in the genus Enchelys. Maupas[5] in 1888 recognized the genus Spathidium of Dujardin and the species spathula of Müller, and thus established the modern name of the organism, Spathidium spathula. The most recent work on the structure and the first on the

[1] O. F. Müller, Vermium terrestrium et fluviatilium historia, 1773, p. 38. Animalcula Infusoria fluviatilia et marina, 1786, p. 40, pl. V, fig. 19.

[2] C. G. Ehrenberg, Abhandl. der Akad. d. Wissensch. zu Berlin, 1830, S. 42; 1831, S. 105. Die Infusionsthierchen als volkommene Organismen, 1838, S. 312.

[3] F. Dujardin, Histoire naturelle des Zoophytes. Infusoires, 1841, p. 458.

[4] F. Stein, Der Organismus der Infusionsthiere, 1859, I. Abtheilung, S. 80.

[5] E. Maupas, Sur la multiplication des Infusoires ciliés, Arch. de Zool. Exp. et Gen., 1888, (2) 4, p. 246.

life-history of Spathidium was done by Moody[6] in 1912, and her study is accordingly the point of departure for the discussion of our own observations.

MATERIAL AND METHODS

The original animal of the pedigree cultures which we are conducting was isolated on November 5, 1920, from a jar of decaying vegetable debris collected at New Haven. Up to the present time a large number of separate lines have been started from the original one, some in which conjugation has been prevented and others in which it has been allowed to occur.

The various pedigreed lines have been carried on depression slides kept in large moist chambers. The culture medium, which was supplied daily at the time of isolation, consisted of two drops of standard beef extract[7] plus a drop of hay infusion which had previously been seeded with small ciliates, chiefly Colpidium. The bacteria which developed afforded food for the small ciliates and these in turn provided food for the Spathidia.

The normal living animals, specimens stained intra vitam, preserved total preparations, and serial sections have been studied both with the monocular and binocular compound microscope with various lens systems, including the Zeiss 2-mm. apochromat, and compensating oculars 4 to 18. The Zeiss binocular dissecting microscope, with lens combinations affording magnifications up to 172 diameters, has also been employed.

For fixation, Schaudinn's solution (two parts saturated solution of corrosive sublimate and one part of absolute alcohol) proved most satisfactory for general cytological studies. In special cases osmic-acid vapor, Zenker's fluid, corrosive-acetic, Worcester's fluid, picric acid, and various other fixatives were utilized. A wide range of stains was used, many of them intra vitam. We may mention Lyon's blue, methylene blue, methyl

[6] J. E. Moody, Observations on the life history of two rare ciliates, Spathidium spathula and Actinobolus radians. Jour. Morph., 1912, vol. 23, p. 349.

[7] L. L. Woodruff and G. A. Baitsell, The reproduction of Paramecium aurelia in a 'constant' culture medium of beef extract. Jour. Exp. Zoöl., 1911, vol. 11, p. 135.

green, nigrosin, dahlia, Bensley's mitochrondria, gentian violet, Loeffler's, Mallory's triple stain, picro carmine, Delafield's haematoxylin, and Heidenhain's iron haematoxylin.

MORPHOLOGY OF SPATHIDIUM

The general structure of Spathidium as described by Moody is corroborated by the study of the animals of our pedigree cultures. The flask-shaped form of a typical vegetative individual is well illustrated in figure 9. But specimens immediately before and after division, encystment, and conjugation, as well as when starved and after a heavy meal, exhibit very marked changes from the typical condition. In addition to the diversities in the relative proportions of the various parts of the cell under these varying circumstances the contortions of the organism, when it becomes entangled in zoogloeal debris, etc., augment the protean picture which it presents, though we have never seen a specimen 'double its length' as recorded by Moody. But such a succession of forms is apparent that many might be—indeed, some have been—designated as distinct species (Cf. figs. 9, 10, 13, 14, 19, and 20). As a matter of fact, the attempt to determine the specificity of widely different specimens in a wild culture led to the discovery of the peculiar advantages afforded by Spathidium for experimental purposes.

Cilia. The ciliation of the specimens in our cultures agrees with Moody's description, there being about sixteen longitudinal rows of cilia evenly distributed over the cell. However, each of these bands is in turn made up of two closely opposed rows of cilia. It has been impossible to demonstrate this composite character of the bands in living specimens, but sections clearly reveal a double row of basal granules in each band. At the edge of the truncated anterior end there are two rows of somewhat longer and stouter cilia, the stronger beat of which is partially responsible for the characteristic gyrations of the anterior end as the organism swims forward revolving on its long axis.

The cytostome consists of a long narrow depression extending almost, if not quite, the whole length of the truncated end nearly at right angles with the long axis of the cell. At the bottom of

the depression there is a slit-like opening into the endoplasm. It is impossible to determine the limits of the opening except when the animal is starting to feed, since at other times the edges are closely appressed. However, when a small ciliate is seized, it rapidly opens and the prey is forced in by means of the contractile edges of the cytostome, assisted apparently, in the earliest stages, by the activity of the larger cilia in this region.

Trichocysts. Maupas and Moody describe a row of minute rod-like bodies near the edge of the peristome. Maupas gives no details in regard to these bodies, apparently taking it for granted that they are trichocysts. But Moody definitely states that "Three artificial methods were used to explode the trichocysts: exposure to osmic acid vapor, treatment with a 2 per cent solution of acetic acid and a solution of methyl green. After treatment with any one of these reagents, in fifty individuals examined, the cilia were found freely extended, the trichocysts being readily distinguished from the long oral cilia inasmuch as they were straight and stiff in appearance, whereas the cilia showed a wavy outline." This statement seems to be sufficiently positive to settle the question, though farther on in the same paragraph the author states that she has not "found the greatly elongated trichocysts of complicated structure described by Maier, Schuberg, and Schewiakoff for Frontonia, Paramecium and other ciliates; owing, however, to the paralyzing effect on Colpidium following contact with the anterior end of the body, I think it safe to interpret, as trichocysts, the short opaque rods so plainly visible in the thickened rim of the mouth."[8]

We have been unable to demonstrate the extrusion of trichocysts in our material, although we have tried all the methods which are effective in other ciliates, such as Paramecium, including the reagents used by Moody herself. In favorable specimens the basal granules of the oral cilia can be seen and present somewhat the appearance of 'trichocysts' as shown in Moody's figure 12. However, they are identical in structure with the basal granules of the smaller cilia on the general surface

[8] Loc. cit., p. 356.

of the cell. Accordingly, since living animals, studied under the highest powers and manipulated with the Barber microdissecting apparatus, stained total mounts, and serial sections fail to reveal the presence of trichocysts, we are forced to conclude that they are not present.[9]

Although trichocysts were discovered by Ellis[10] as long ago as 1769, given their present name by Allman[11] in 1855, and supposed to be 'poison organs' by Lachmann[12] two years later, there is to-day little conclusive data in regard to their function. Even in forms like Paramecium and Frontonia with obviously highly developed trichocyst apparatus, so far as we are aware, the function of the trichocysts is chiefly a matter of assumption. Mast clearly showed that the trichocysts of neither of these organisms have any paralyzing effect on Didinium, and attributed to them merely a mechanical effect in that they form a viscid mass about the organism which hampers its enemy. Indeed, Jennings stated: "It is possible that the discharge (of trichocysts) is really an expression of injury,—a purely secondary, even pathological phenomenon, like the formation of vesicles on the surface of an injured specimen."[13]

On the authority of Balbiani,[14] trichocysts were stated to occur in Didinium until Thon,[15] corroborated by Mast,[16] demonstrated their absence. However, Thon believed that Didinium has a paralyzing effect on a Paramecium which has been struck by the

[9] L. L. Woodruff and Hope Spencer, The food reactions of the infusorian Spathidium spathula. Proc. Soc. Exp. Biol. and Med., 1921, vol. 18, p. 183.

[10] J. Ellis, Philosophical Trans. Royal.Society, 1769, vol. 59, p. 144.

[11] G. J. Allman, On the occurrence among the Infusoria of peculiar organs resembling thread-cells. Quart. Jour. Microscopical Science, 1855, vol. 3, p. 177.

[12] J. Lachmann, Annals and Magazine of Natural History, 1857, vol. 19, p. 126.

[13] H. S. Jennings, Behavior of the lower organisms, 1906, p. 91.

[14] E. G. Balbiani, Observations sur le Didinium nasutum. Arch. d. Zool. Exper. et Gen., 1873, T. 2, p. 363.

[15] K. Thon, Ueber den feineren Bau von Didinium nasutum. Archiv f. Protistenk., 1905, p. 289.

[16] S. O. Mast, The reactions of Didinium nasutum (Stein) with special reference to the feeding habits and the function of trichocysts. Biol. Bull., 1909, vol. 16, p. 91.

seizing organ. Calkins[17] apparently showed that there is such an effect, and in the absence of trichocysts interpreted certain deeply staining 'granular striae' near the apex of the trichites comprising the seizing organ as representing the seat of the poison. Actinobolus radians also may be mentioned; both Erlanger and Calkins[18] held that this ciliate captures Halteria grandinella by means of trichocyst-tipped tentacles. But Moody's study of Actinobolus reduced the 'trichocysts' to 'dark granular trichocyst material.'[19]

There is nothing comparable in structure nor, so far as has been proved, in function between trichocysts, in the original sense of the term, and the 'offensive trichocysts' described by Maupas, the 'needleform trichocysts' of other authors, or 'trichocyst material' of still others. Although in the present state of knowledge the line of demarcation is ill defined in many cases between bodies which are purely supporting in function, others which possibly are both supporting and offensive or defensive, and lastly true trichocysts, only confusion is served by extending the significance of the word trichocyst, which in sensu strictu has a very definite connotation, to widely divergent structures.[20]

Trichites. Although trichocysts are not present in Spathidium, there are slender rodlets imbedded like a paling in the thickened rim of the anterior end of the cell which give it a striated appearance under certain conditions. These apparently are what Moody (fig. 18) interprets as parallel thickenings of the cortex which reinforce the pharynx. As a matter of fact, the rodlets may be identified as trichites, provided the use of this term does not necessarily imply that they are the seat of the poison. In addition to the trichites in the oral region, it is possible to identify individual trichites distributed in the endoplasm of the cell. Some

[17] G. N. Calkins, Didinium nasutum: I. The life history, Jour. Exp. Zoöl., 1915, vol. 19, p. 225.

[18] G. N. Calkins, The Protozoa, 1901, p. 50.

[19] Loc. cit., p. 372.

[20] E. Maupas, Contribution à l'Étude Morphologique et Anatomique des Infusoires Ciliés. Arch. d. Zool. Exper. et Gen., 1883, (2), 1, p. 611. Y. Delage and E. Hérouard, La Cellule et les Protozoaires, Paris, 1896, p. 434.

of these apparently are newly formed and being transported to the oral region, while others may well be trichites which have been torn away during the process of prey ingestion (fig. 12).

In view of the fact that this organism paralyzes its prey, it is natural to seize upon the chief structural differentiation of the oral region as the locus of the poison. But on account of the problematical function of clearly defined trichocysts when present, and the absence of any proof that the trichites of Spathidium are actually the seat of the poison, we prefer not to prejudge the question. Though, it is true, Blochmann in particular has interpreted the trichites of certain other ciliates as related to poison production. The trichites of Spathidium are apparently the 'Tastkörperchen,' identified by Stein in an organism which probably is a Spathidium, and the 'trichocysts' noted by Maupas.

The trichites of Spathidium obviously are comparable to the rods which form a framework for the mouth in many of the more generalized gymnostomatous ciliates and undoubtedly are homologous with the strands comprising the highly specialized seizing organ of Didinium. Thon believed that in Didinium he could discriminate between trichites and 'Stäbchen des Reusenapparates,' but no such distinction is possible in Spathidium. The paling of trichites suggests that the whole surface of the truncated anterior end of Spathidium is homologous with the cytostome of such a form as Enchelyodon. In other words, the mouth proper of Spathidium is really the depressed area bounded by the slightly thickened edges of the anterior end of the animal. It is always open and leads to the slit-like cytopharynx immediately below, the sides of which remain appressed except when food is being ingested. This interpretation also seems to be supported by the method by which food is engulfed, which involves a grasping and 'mouthing' of the quarry by the region bearing the trichites.

Contractile vacuole. The Spathidia of the cultures under consideration characteristically possess one large contractile vacuole situated at the posterior end of the cell, though under certain undetermined conditions animals now and then appear with two smaller, closely opposed contractile vacuoles in the same situation.

But sooner or later the two merge into one large vacuole. In this regard our animals are exactly similar to those in Moody's cultures.

Nuclei. The nuclear apparatus of Spathidium as exhibited by our animals is remarkably variable, though it agrees, in the main, with the description given by Maupas. The typical vegetative cell apparently contains two long ribbon-like macronuclei which extend nearly the whole length of the cell (fig. 11). However, owing to the intertwining of these bands, we have been unable to decide whether they are actually distinct or whether they are united at the posterior end to form a single structure. It seems probable that both conditions occur because the macronucleus varies considerably from day to day; very many otherwise typical animals showing the macronucleus fragmented into a few or many separate pieces. Thus far it has not been possible to associate these morphological macronuclear changes with characteristic events in the life-history.

Maupas states that there are from six to nine micronuclei present in Spathidium and the same is apparently true for the organisms of our culture. Seven are visible in the animal illustrated in figure 11. In the majority of cases they are difficult or impossible to identify owing to the fragmented condition of the macronuclear apparatus and the presence of remnants of the nuclei of ingested Protozoa.

Moody was unable to discover any micronuclei in the animals of her culture and states: "Although it is generally thought that the possession of two types of nuclei is characteristic of the ciliates, the present observations give no evidence of this differentiation in Spathidium."[21] In this connection it is important to note that Moody says: "Conjugation was not observed, though numerous attempts were made to bring it about." From the standpoint of the micronucleus, then, Moody's animals were characteristically different from those of our cultures, which not only are micronucleate, but the micronuclei function in a typical manner during conjugation. Indeed, the chief value of the animals of this culture is the ease with which they may

[21] G. N. Calkins, The Protozoa, 1901, p. 358.

be induced to conjugate.[22] Moody's amicronucleate race is undoubtedly comparable with the amicronucleate races of various ciliates recently described by Dawson,[23] Landis,[24] Woodruff,[25] and Patten.[26]

FEEDING BEHAVIOR

Spathidium is one of the so-called hunter ciliates, employing for food the smaller ciliates which it captures during its perigrinations. Maupas, in his study on the life-history of Infusoria, carried for a short time a culture of this organism which he fed chiefly on Glaucoma, Cyclidium, and Cryptochilum. Moody found it impossible to conduct her long pedigree culture without Colpidium for food and definitely states that she never saw any other ciliates paralyzed or eaten. The animals of our culture readily paralyze and swallow almost any small ciliate with which they come in contact, though flagellates of various kinds are immune. As a matter of fact, however, we have employed chiefly species of Colpidium for food because they can be developed for the purpose in countless numbers so readily in small flasks of hay infusion.

Spathidium typically swims forward quite rapidly through the water, revolving on its long axis, and gives at first glance the impression that it is exploring its path with its truncated anterior end. This appearance results chiefly from the fact that the axis of revolution does not pass directly from posterior to anterior end, but leaves the body a short distance posterior to the mouth region. Accordingly, the narrower anterior end traces the circumference of a circle of greater diameter than the rest of the body, especially when the animal is swimming

[22] L. L. Woodruff and Hope Spencer, The early effects of conjugation on the division rate of Spathidium spathula. Proc. Soc. for Exper. Biol. and Medicine, 1921, 18. The survival value of conjugation in the life history of Spathidium spathula. Ibid, 1921, 18.

[23] J. A. Dawson, An experimental study of an amicronucleate Oxytricha. I, Jour. Exp. Zoöl., 1919, vol. 29, p. 473. II, ibid., 1920, vol. 30, p. 129.

[24] E. M. Landis, Amer. Naturalist, 1920, vol. 54, p. 453.

[25] L. L. Woodruff, Jour. Exp. Zoöl., 1921, vol. 34, p. 329.

[26] M. W. Patten, Proc. Soc. for Exper. Biology and Medicine, 1921, vol. 18, p. 188.

rapidly. In addition, the narrow anterior end is quite flexible, and this makes the behavior still more complex.

A careful analysis of the reactions of Spathidium to various stimuli reveals quite clearly that in general its behavior can be interpreted in accordance with the 'avoiding reaction' as worked out for various Protozoa by Jennings. In most cases when a Spathidium, which is swimming forward, is stimulated, it reverses the effective stroke of the cilia, moves back for a short distance, turns toward the side with the shorter anterior tip, and then proceeds forward again on a slightly different course. So far its behavior appears quite stereotyped. But not so when the anterior end strikes a small ciliate. Spathidium now exhibits a series of reactions which may well be fundamentally of the same nature as its others, since Spathidium is limited, of course, by the means at its command, but no good purpose will be served by attempting to interpret it in the same terms.

A casual survey of a number of hungry Spathidia swimming among Colpidia gives one the impression that the Spathidia chase their prey as one tiny ciliate after another falls victim to the carnivores—one of the most spectacular sights in the world of the Infusoria. But a careful study soon makes it clear that the captures are the result of the chance contact of the oral region of a Spathidium with a small ciliate. Indeed, when attempting to follow the movements of a single Spathidium through a heavy culture of Colpidia in order to study the details of the swallowing process, one is frequently astounded to find how easily they miss their prey! Nothing happens unless the quarry strikes the anterior end of the Spathidium nearly or quite in the center. Touching the edges produces no effect on either animal.

But when a Colpidium or similar ciliate is fairly hit, the picture changes instantly with respect to both of the animals involved. The prey, which up to this moment was swimming rapidly, usually instantly becomes motionless and quickly shows pathological changes involving vacuolization of the cytoplasm and disintegration of the cilia. Sometimes, however, paralysis and death are not quite so rapid, and under these conditions the quarry appears to tremble and moves haltingly away for a short

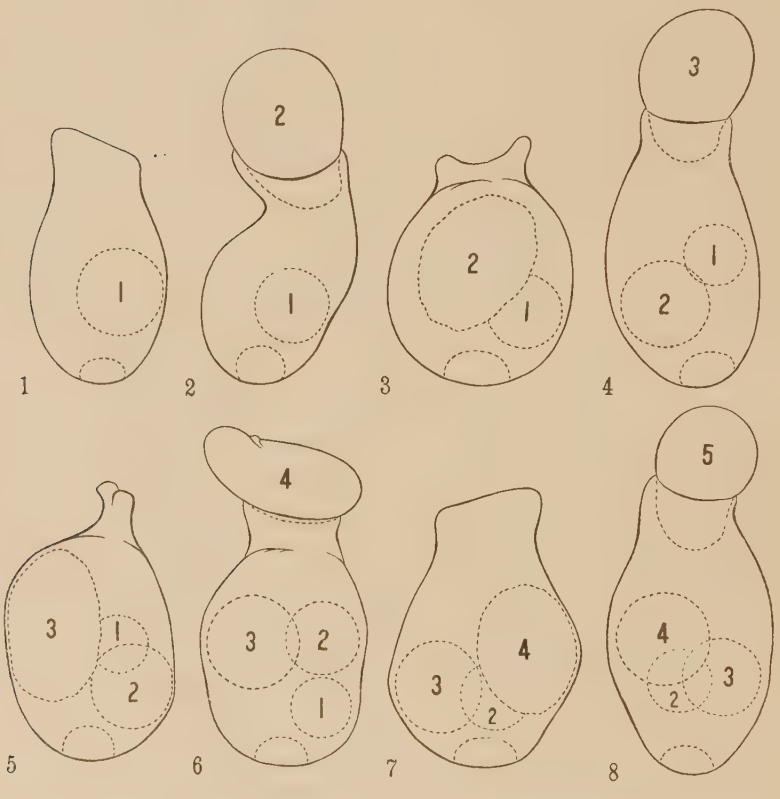
distance. The behavior of Spathidium differs markedly under these two conditions.

If the prey is instantly paralyzed it frequently remains against the oral region of its captor and the reactions of the latter are then relatively simple. The stimulus afforded by the contact immediately stops the forward movements of the Spathidium, which gives a few rapidly repeated avoiding reactions that tend to keep it at or near the spot where the capture was made. Meanwhile, the prey is moved around, apparently as a result of the combined activity of the longer oral cilia and the gradually expanding edges of the truncated end of the Spathidium, until it may conveniently be encircled. This process of mouthing successfully accomplished, nothing further is visible except the gradual sinking of the prey through the greatly expanded cytostome of the captor into the endoplasm (figs. 17, 18, and 19). From the point of ingestion the course of the food vacuole within the cell may be readily followed, especially when the prey has previously been fed with powdered carmine (cf. figs. 1 to 8). The whole process from contact to complete ingestion takes place in about thirty seconds, and the Spathidium, which usually sinks to the bottom during the process, within a few seconds more resumes its active foraging. Three such captures have been observed to occur within eight minutes. Even certain conditions which must be considered as highly abnormal apparently interfere not at all with food taking. For example, a Spathidium transfixed to a cover-glass with the needle of a Barber microdissection apparatus will paralyze a Colpidium and swallow it, provided, of course, the latter remains in contact with the mouth of the Spathidium.

Although the behavior of Spathidium is characteristic and remarkable when it makes a direct capture, the most interesting and significant cases are those in which either the prey is not instantly paralyzed, so that it moves a short distance away, or, though rendered motionless, becomes removed from the oral region of the Spathidium. Under these circumstances, the factors involved obviously become highly complex and the behavior of the Spathidium is not identical in any two cases. The study

of a large number of such captures, however, justifies, we believe, the following general description.

When the prey fails to remain in contact with the oral region, the Spathidium immediately gives a series of avoiding reactions, which, unlike those that occur when the prey remains in contact, tend to increase in extent so that the organism not only remains in the general region in which the attack occurred, but



Figs. 1 to 8. Food-taking by a single specimen of Spathidium spathula. 1. Animal when first observed. Contains one food vacuole enclosing a Colpidium (*1*). The contractile vacuole is shown at the posterior end of the Spathidium in all the figures. 2. Seizing a second (*2*) Colpidium. 3. Second Colpidium enclosed in food vacuole. 4. Third Colpidium being engulfed. 5. Third Colpidium in food vacuole. 6. Seizure of fourth Colpidium. 7. Fourth Colpidium within vacuole; food vacuole with first Colpidium no longer discernible. 8. Engulfing of fifth Colpidium. Observations, extending over about an hour, and drawings by Miss J. Elizabeth Lovett, artist to the Osborn Zoological Laboratory.

also moves backward and forward in the neighborhood of its prey. It is obvious, of course, that such reactions sooner or later will again bring the mouth of the Spathidium in contact with the food. But, a careful analysis of many cases clearly shows that usually these stereotyped reactions are insufficient to account for the comparative precision exhibited by the Spathidium in recovering its quarry. And further, the reactions of the Spathidium are successively modified so that, although it may have become removed several times its own length, within a relatively short time the oral region again comes in direct contact with the food, which is then engulfed in the manner previously described. This is a fact that we believe is indisputable. Of course, such a result as the above does not invariably occur; sometimes the prey is never found again. But, when all the complex factors involved are appreciated, it is remarkable how few paralyzed ciliates are lost.

A Colpidium before being paralyzed has no effect whatsoever on Spathidium; after paralysis it appears to be a center of attraction, and not only to the Spathidium whose oral region it touched, but also in a much less marked though significant degree to other Spathidia in the immediate neighborhood. This last observation naturally suggests the probable explanation. Chemical substances excreted by the dying organism, or secreted at the time of contact by the captor itself, afford a stimulus which results in the characteristic behavior of Spathidium. It is probable that the effective substance in question is secreted by the Spathidium itself because the oral region of the latter is frequently, especially after an unsuccessful capture, a stimulus to neighboring specimens. And the same factor may well be responsible for the peristomal fusion incident to conjugation. Whatever the explanation may be, the point not to be lost sight of is that Spathidium 'senses at a distance' its paralyzed prey.

The reaction against the early anthropomorphic interpretations of the behavior of the Infusoria resulted in a conception of the simpler animals which almost left out of account the fact that they are organisms. The study of Spathidium adds one more to the body of data being rapidly brought to the fore that makes

it increasingly difficult to interpret Protozoan behavior in simple terms.

SUMMARY

The essential points presented are:

1. Spathidium does not possess trichocysts, and consequently the extrusion of such structures is not responsible for the paralysis of the prey.

2. Trichites are present in the oral region, and form a paling which is apparently comparable with the supporting rods about the mouth in the more generalized gymnostomatous ciliates. There is no conclusive evidence that the trichites are the locus of the poison.

3. Spathidium spathula, in our cultures, possesses clearly defined micronuclei. Therefore, the Spathidium studied by Moody must be interpreted as an amicronucleate race.

4. The food of Spathidium is limited to small ciliates, but not to members of the genus Colpidium.

5. Spathidium comes in contact with its prey solely through chance.

6. Prey which has been paralyzed and has become removed from the oral region of the Spathidium is recovered in a majority of instances by a complex series of successively modified reactions, indicating 'sensing at a distance.'

7. The factor involved in the 'sensing at a distance' is apparently a substance secreted by the Spathidium when the prey is paralyzed.

May 6, 1921.

PLATE

PLATE 1

EXPLANATION OF FIGURES

9 Typical foraging Spathidium.

10 A common form when food is scarce.

11 Optical section of total mount, stained with Delafield's haematoxylin, to show macronuclear and micronuclear apparatus. Seven micronuclei are present, one at the posterior end above the contractile vacuole.

12 Section (4μ) to illustrate the anterior paling of trichites and the trichites distributed through the endoplasm; nuclear apparatus, etc., omitted. Heidenhain's iron haematoxylin.

13 Form of Spathidium in an early stage preparatory to encystment.

14 Form shortly before encystment.

15 Spathidium dividing.

16 Division nearly completed.

17 to 19 Spathidium swallowing a Colpidium. Successive stages.

20 Form shortly after division or when quieting down preparatory to encystment.

Drawn by Miss J. E. Lovett.

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Tertiary Spiders and Opilionids of North America

BY

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TERTIARY SPIDERS AND OPILIONIDS OF NORTH AMERICA

INTRODUCTION

The present study is based on collections placed at the disposal of the author through the courtesy of the Museum of Comparative Zoology of Harvard University, of the U. S. National Museum, of the American Museum of Natural History, of Professor W. H. Twenhofel of the University of Kansas and of Professor T. D. A. Cockerell of the University of Colorado. To the above institutions and gentlemen the author expresses his deep appreciation of the privilege thus accorded him not only to reinvestigate the types previously described by Scudder, but to add several new species to those already known. The author is also deeply indebted to Professor Charles Schuchert who was instrumental in obtaining most of the material above mentioned. Although the study of the specimens was completed some time ago the work was considerably retarded at first by extraneous activities of the author during the war time and later by the difficulty of getting the proper help in making the drawings. All attempts to photograph the material proved highly unsatisfactory. Only a few specimens come out sufficiently well to allow of reproduction. The majority of the specimens are so faint and their colour so much like that of the surrounding rock that no combination of rayfilters, plates and illumination systems is of help in differentiating the specimens from the rock. At the same time the specimens are sufficiently well preserved to be clearly seen, measured and drawn. Thus drawings proved in every way much more satisfactory than photographs and were prepared by Miss F. E. Lovett, Mr. H. D. Rhynedance and myself. The pictures given by Scudder on Plate XI of his stately volume on Tertiary Insects of North America are so good that the expense of redrawing them would not have been justified and the reader is referred to Scudder's work.

The collections above mentioned contain altogether two distinct and hitherto unknown species of Opilionids and forty-seven distinct species of spiders representing seventy-six specimens or 89 pieces since several specimens consist of two pieces each. Six-

teen species of spiders are new to science and are described here for the first time. Besides the more or less complete specimens the Scudder collection of the Museum of Comparative Zoology contains also four impressions of single legs and eight cocoons. The latter were described by Scudder as belonging to a species which he named *Aranea columbiae*. I have not seen these specimens, nor do I include them in the present study because of the utter uncertainty of classification. That the specimens in question are spider-cocoons seems to be if not absolutely beyond doubt, at least highly probable. But even the family could not be identified with any degree of certainty, and the figures given by Scudder in his Tertiary Insects on Plate 2 make one believe that the cocoons in question are not all of the same species.

A few words of explanation are necessary concerning the recognition of species. Generally speaking it is much easier to recognize in a new palaeontological specimen a new species than to place it correctly in the system. Our knowledge of American Tertiary spiders was based on Scudder's work. A keen observer and a man broadly versed in the systematics of Arthropods, Scudder had no difficulties in assorting his material and describing the new species. He made minor mistakes, in a few cases he placed in the same species specimens distinctly belonging to different species, but the bulk of his species stand good to-day. At the same time Scudder has never been a professional arachnologist and the principles of classification gradually arrived at through the work of several distinguished European arachnologists remained unknown to him or did not appeal enough to him to be extended from the field of recent forms to that of extinct ones. Therein lies the weakness of Scudder's work. For although his species are good, the characters upon which their description is based, from the point of view of modern knowledge are entirely inadequate for the purpose of placing the species in the system. As will be pointed out further down, not only the generic characters, but even family characters as well as characters of entire sub-orders are usually so obliterated that Scudder had to rely mostly on external similarity. Naturally species of the same family have more resemblance to each other than to species of other families. External resemblance is the first principle in any assortment of large collections. At the same time, as is well known, it is one of

the most misleading methods imaginable. In spiders this is particularly true. We have parallel lines of development in Theraphosid and true spiders and among the latter in cribellate and ecribellate spiders. The similarity is so great that for years *Uloborus* and *Hyptiotes* of the Cribellates were placed among the orbweavers of the Ecribellates. The Agelenids have their counterpart in Diplurids among the Theraphosids. The similarity between our common *Agelena naevia* and *Evagrus mexicanus* of the Diplurinæ is so striking that it extends over their behaviour and webconstruction, although *Evagrus* is more than twice the size of *Agelena* and on closer examination possesses all the distinctive characters of the group to which it belongs. Cases are known where external similarity has woefully misled the systematist. Thus a larva of a recent fly belonging to the genus *Microdon* has originally been described as a hard mollusk, and the cases constructed by some recent caddis-fly larvæ as shells. Misled by external similarity, the distinguished arachnologist Count Keyserling described a new genus of a cribellated spider, *Acanthoctenus* with two species as a new genus of the sub-order Citigradæ, because he did not pay attention either to the spinnerets or the metatarsus of the fourth leg. The synonymy contained in arachnological works demonstrates clearly that similar mistakes have been made repeatedly by most eminent arachnologists. It only shows that errors are very easy to make and that in the absence of most important characters such errors are inevitable. Under the circumstances a modern arachnologist may well condemn any attempt at classification of extinct spiders. In recent spiders it is a comparatively easy matter to place a specimen in its proper family, it is more difficult to assign it to a genus, and it requires often minute study of characters invisible to the naked eye to determine the species. In this respect the study of extinct forms practically reverses the difficulty. With few specimens in existence it is a comparatively simple matter to establish the fact that a specimen under observation belongs to a different species from those already known; but with very few exceptions it is absolutely impossible to place it correctly in the system. What then should be done? Obviously the species should be carefully described and provisionally placed in such a genus and family with the recent representatives of which it has most resemblance.

When it comes however to a comparison between American and European spiders extreme caution is necessary. With families and genera uncertain, what reliance can be placed upon deductions made from such a comparison? Scudder may be pardoned for his attempt to do it, but no excuse would be valid in the case of a man thoroughly versed in the modern systematics of recent spiders. Faunistic and climatic conjectures are therefore entirely out of place.

Another side of the investigation was also sadly disappointing to the author, namely the complete absence of any structures which would allow one to unravel the mystery of the origin of the method of reproduction peculiar to spiders. In those cases where the male palpi are well preserved they show that at that geological period the copulatory apparatus was already fully developed and occupied the same position as in recent spiders. In one case the epigynum of a female is also surprisingly well preserved and quite of the type found in recent species.

THE RELATIONSHIPS OF ARACHNIDA.

It may be not quite out of place here to consider first of all the relationships of Arachnida and their place in the System of Arthropoda. Already in my monograph of Palaeozoic Arachnida I have pointed out the reasons why Pycnogonida, Tardigrada and Pentastomida (Linguatulida) have no place among the Arachnida. On the other hand I have included under Arachnida the Gigantostraca and the Xiphosura (Limuloids). I have also given a definition of the Class Arachnida in conformity with our knowledge at that time. Since then I have done some extensive study of segmentation in Arthropods, part of which studies is in press at this moment. The readers interested in the subject are referred to this paper which will appear in the Journal of Morphology. But the results of the investigation may be briefly summarized as follows: The Lankesterian division of the body of an Arachnid into a prosoma, mesosoma and metasoma of six segments each is entirely untenable and must be dropped. The Xiphosura have much in common with the Arachnida, but also possess many features which make their relationship with the Arachnida more than doubtful. Their arterial circulatory system is perineural. Their

carapace represents apparently not a fusion of the head with the thorax alone, but includes the first and second abdominal tergites. The segmentation of the scorpion shows the appearance of a spurious segment, but is in other respects similar to that of spiders. The carapace in all Arachnida represents the fusion of the tergites of the head and thorax, unless one or more of the thoracic somites are free, but it never includes any of the abdominal tergites. The arterial system is entirely independent of the nervous system and composed of supraneural, subneural and interneural arteries. Xiphosura cannot be regarded as ancestors of Arachnida for the same reasons. Very likely Xiphosura have Trilobites for ancestors as is also suggested by Raymond in his extensive monograph. The relationship of the Gigantostraca (Eurypterida) cannot be determined at present without further research. Provisionally at any rate they may be safely left with the Arachnida.

The new definition of the Class Arachnida may therefore be given in the following words. Arachnida are Arthropoda with heteromeric segmentation. Their body is composed of 21 somites four of which are prae-oral in the adult, the fourth somite bearing the chelicera. The first postoral somite belongs to the head and bears the pedipalpi. The thorax consists of four somites each provided with a pair of appendages, with the exception of some Acari in which only the first and second thoracic somites bear appendages. Head and thorax are usually fused into a cephalothorax, but in some Orders one, two or even three of the thoracic somites may remain free. The abdomen is composed of 12 somites, the first of which is rudimentary in the adult, being represented only by its neuromere, except in the Pedipalpi in which it persists throughout life. Further fusion of somites involves the posterior end of the abdomen, beginning with the last somite and proceeding forward. Secondary spurious segmentation in the abdomen, as in the case of the 18th somite of the scorpion, may result in the formation of an additional segment which does not possess the value of a somite. The anus is always on the last somite. The genital opening is on the eleventh (second abdominal) somite. Nephridia modified as coxal glands situated in the thorax. Respiration by means of lung-books or tracheæ, in some forms organs of respiration entirely absent. Circulatory system with a heart in which the cardio-aortic valve always marks the

limit between the last thoracic and first abdominal somite (i. e. between the 9th and 10th somites). The reduction of the heart always begins at the posterior end and proceeds forward. The arterial system wherever fully developed consists of an aorta with its arch and pedal arteries and a series of interneural arteries connecting the supraneural with the subneural artery. The legs are typically of 7 joints the last of which in some cases may be subarticulated; the pedipalpi of 6 joints, the terminal joint corresponding to the last two joints of the legs. The cheliceræ are 2 or 3 jointed. The eyes whenever present are always simple, not more than 12 in number and belong to the first and second somites. External segmentation of the adult does not coincide with true segmentation.

To facilitate the understanding of the above definition the following table comparing apparent segmentation in several Orders of Arachnida with true segmentation may not be without practical value to students of extinct Arthropoda.

The Table needs little explanation. The transverse lines indicate the limits between segments as they actually appear to the eye, not as derived from the presence of appendages or from the study of internal anatomy and embryology. The somitic value of the sternum in the cephalothorax is omitted in all cases in order to emphasize the differences in the composition of the carapace as well as for lack of space. Attention is called to the fact that the normal or most primitive type of segmentation is found not in Scorpions but in Pedipalpi. There is still some difference of opinion concerning the eyes in Araneæ. Schimkewitsch and Scheuring homologize the indirect eyes of spiders with the median eyes of scorpions, and the direct eyes with the lateral eyes of scorpions. Schimkewitsch even gives a figure of the ganglia as derived from the study of sections in embryos of *Ischnocolus*. It seems to me however that the evidence adduced by the above mentioned investigators is not sufficient to decide the case in their favor and I retain for the present the homology of the median eyes of the scorpion with the direct eyes of spiders.

It is not impossible that Arachnida possessed originally a few somites *in front* of the eyes. Corresponding neuromeres have been figured by Schimkewitsch. If such is the case the body of an Arachnid is in reality composed of more than 21 somites, but

	SOMITES	SCORPIONES		PEDIPALPI		SOLIFUGAE	
CEPHALON	1 (1 C)	CARAPACE	MEDIAN EYES	CARAPACE	MEDIAN EYES	CARAPACE	MEDIAN EYES
	2 (2 C)		LATERAL EYES		LATERAL EYES		LATERAL EYES
	3 (3 C)		ROSTRUM		ROSTRUM		ROSTRUM
	4 (4 C)		CHELICERAE		CHELICERAE		CHELICERAE
	5 (5 C)		PEDIPALPI		PEDIPALPI		PEDIPALPI
THORAX	6 (1 TH)		1 pair of LEGS		1 pair of LEGS		1 pair of LEGS
	7 (2 TH)		2 pair of LEGS		2 pair of LEGS		2 TERG. = 2 p. LEGS = 1
	8 (3 TH)		3 pair of LEGS		3 pair of LEGS		3 TERG. = 3 p. of LEGS
	9 (4 TH)		4 pair of LEGS		4 pair of LEGS		4 TERG. = 4 p. of LEGS
ABDOMEN	10 (1 A)	ABDOMEN	(First abdominal neuromere)	ABDOMEN	1 TERGITE = STERNITE	ABDOMEN	?
	11 (2 A)		1 TERGITE = GENITAL OPERC.		2 TERG. = GEN. OP., 1 LUNG		1 Segment = GENITAL O
	12 (3 A)		2 TERGITE = COMBS		3 TERGITE = STERN., 2 LUNG		2 SEGMENT = 2 STIGM.
	13 (4 A)		3 TERGITE = 1 LUNGS		4 TERGITE = STERNITE		3 SEGMENT = 3 STIGM.
	14 (5 A)		4 TERGITE = 2 LUNGS		5 TERGITE = STERNITE		4 SEGMENT = MEDIAN
	15 (6 A)		5 TERGITE = 3 LUNGS		6 TERGITE = STERNITE		5 SEGMENT
	16 (7 A)		6 TERGITE = 4 LUNGS		7 TERGITE = STERNITE		6 SEGMENT
	17 (8 A)		7 TERGITE = STERNITE		8 TERGITE = STERNITE		7 SEGMENT
	18 (9 A)		1 CAUDAL SEGMENT 2 CAUDAL SEGMENT		9 TERGITE = STERNITE		8 SEGMENT
	19 (10 A)		3 CAUDAL SEGMENT		1 CAUDAL SEGMENT		9 SEGMENT
	20 (11 A)		4 CAUDAL SEGMENT		2 CAUDAL SEGMENT		10 SEGMENT
	21 (12 A)		5 CAUDAL SEGMENT		3 CAUDAL SEGMENT		

	OPILIONES	ARANEAE	LIMULUS
CARAPACE	EYES	DIRECT EYES	MEDIAN EYES
	?	INDIRECT EYES	LATERAL EYES
	ROSTRUM	ROSTRUM	? ROSTRUM
	CHELICERAE	CHELICERAE	CHELICERAE
	PEDIPALPI	PEDIPALPI	PEDIPALPI
	1 pair of LEGS	1 pair of LEGS	1 pair of LEGS
	2 pair of LEGS	2 pair of LEGS	2 pair of LEGS
	3 pair of LEGS	3 pair of LEGS	3 pair of LEGS
	4 pair of LEGS	4 pair of LEGS	4 pair of LEGS
ABDOMEN	?	PETIOLUS	CHILARIA
	GEN. Arches = GENIT. OPERC.	GENITAL OPENING = 1 LUNGS	GENITAL OPERCULA
	STIGMA	2 LUNGS or SPIRACLE	1 GILL-BOOKS
	1 SEGMENT	CRIBELLUM and ANT. SPINNER.	2 GILL-BOOKS
	2 SEGMENT	MED. and POST. SPINNERETS	3 GILL-BOOKS
	3 SEGMENT	ANAL TUBERCLE	4 GILL-BOOKS
	4 SEGMENT		5 GILL-BOOKS
	5 SEGMENT		6 ABDOMINAL SPINE
	6 SEGMENT		2 postbranchial neurom.
	7 SEGMENT		3 postbranchial neurom.
	OPERCULUM ANALE		

in absence of definite data it seems wiser to limit the discussion of homologies to somites the presence of which can be always demonstrated. The same holds true of Xiphosura.

CLASSIFICATION IN ARANEÆ.

Let us now proceed in detail with the discussion of the problem of classification in spiders and especially with that of identification of extinct species. Lest much of what I have said may seem to be unsupported by facts, arbitrary and attributable rather to personal shortcomings of the author than to poor preservation of the material itself, I shall give two examples and submit these to an exhaustive analysis so that the reader may form a judgment for himself. The spiders which I choose for this purpose, *Parattus resurrectus* and *Titanocca ingenua,* are fairly well preserved. Both belong to the sub-order of Arachnomorphæ or True Spiders, characterized by the direction of the axis of articulation of the chelicerae, the presence of well developed maxillary lobes and the possession of a single pair of lungs and of a single (or of a pair) tracheal spiracle. Neither of these characters can be determined from an examination of the specimens in question, but the combination of other characters, the general appearance, the size, the proportion of the legs, the *"tout ensemble"* so to say, makes a mistake impossible. So far, so good.

The first sub-division of True Spiders is based on the presence or absence of a cribellum in front of the spinnerets and of a calamustrum on the fourth metatarsus. The Family Attidæ, to which according to Scudder who established the genus *Parattus* for three species of Tertiary Spiders that genus belongs, comes under the division Ecribellatæ. The absence of a cribellum and calamistrum in recent spiders may be ascertained without difficulty, but only with the aid of a lens. Moreover, the calamistrum can be seen only in a certain position of the leg. How much of these structures may have been preserved in extinct spiders? Spines and hair are well preserved in some specimens. Spinnerets, as a rule, are not nearly as well preserved. In *Parattus resurrectus* one can see with the aid of a good binocular dissecting microscope at a magnification of about 60 diameters simple brown hair on the spinnerets and similar hair and spines on all legs. No cribellum and no calamistrum are visible. In absence of traces of these

structures we may reasonably assume that *Parattus* did not possess them, but the absence of a character is in itself not a sufficient proof, because in no case are all structures preserved.

Assuming then that *Parattus* actually did not possess these structures we may place it in the division Ecribellatæ. This division is composed of 28 families in recent spiders. What are the distinctive characters of the Family Attidæ? 1) Presence of a *single* spiracle immediately in front of the spinnerets; 2) Two tarsal claws and ungual tufts; 3) Eight eyes in three or four rows, anterior median eyes by far the largest, eyes of second row minute; 4) All eyes of the diurnal type; 5) Clypeus vertical; 6) Absence of colulus; 7) Body often covered with scales. Of these characters the relative size and position of the eyes is the most conspicuous and usually allows at once to recognize an Attid in a recent spider. How about *Parattus?* The eyes of the second row are not discernible in any of the three species. Scudder describes for *Parattus resurrectus* the "eyes of the second row from one-eighth to one-tenth the size of those of the first row, situated behind and within the middle anterior pair, so that lines drawn through the middle of the large and small ones would meet in a right angle behind the small ones and leave them distant from each other by about their own diameter; the outer edge of either of the small ones is behind the inner edge of one of the large ones" (p. 54). No similar position of the eyes of the second row is known to occur in any of the hundreds of recent Attidæ. If the little impressions which Scudder described as eyes of the second row actually represent eyes then *Parattus* is not an Attid at all. Similarly, if the anterior median eyes are equal in size to or scarcely larger than the anterior lateral eyes, then, as Scudder himself points out in his definition of the genus *Parattus,* this character separates *Parattus* "from all members of the Family." But the eyes of the third row being "not discernible" in *Parattus resurrectus,* "doubtful" in *Parattus latitatus* and preserved only in the otherwise poorly preserved specimen of *Parattus evocatus,* in which the eyes of the second row according to Scudder himself are "indiscernible," what value can be placed in the generic character when the corresponding family character is not in evidence?

Of the other six characters of the Family Attidæ enumerated

above, characters the combination of which might have come to our help, not a single one can be recognized. Even in recent spiders the spiracle can be seen only with difficulty, the diurnal type of eyes is an expression of the internal structure and cannot be preserved, the clypeus, if properly preserved would appear only in a plane vertical to the surface of the specimen and the absence of the colulus is only a negative character. The claws could be seen under microscope if they were preserved, but although every spider has either two or three claws on each tarsus, none of the specimens in question show any trace of them. Similarly, although the specimens show well preserved hair and spines, there is no trace of scales. The evidence, such as there is, points therefore *against* the probability of Parattus belonging to the Family Attidæ.

But let us assume for the moment that *Parattus* is an Attid. The separation of Attidæ into genera is based on the following characters:—1) Number and structure of teeth on the lower margin of cheliceræ; 2) Size and shape of the entire eyegroup; 3) Relative position of the eyes of the second row; 4) Number of spines on the underside of the first tibia; 5) Number and distribution of other spines on legs; 6) Distance between the anterior coxæ; 7) Shape and especially elevation of carapace; 8) Shape of underlip; and 9) Shape of sternum. Most of these structures are either poorly preserved or entirely indiscernible. If the family relationship were definite then indeed the genus would be sufficiently characterized by the proportion of the anterior eyes and we could disregard the other characters. As for the specific differences given by Scudder for his three species of *Parattus* and consisting in the shape and proportions of the cephalothorax, these differences in recent spiders would undoubtedly have generic and not specific value. For the recognition of species in Attidæ is based chiefly on the structure of the male palpus and female epigynum, the distribution of color on the abdomen, carapace and legs, the type of hair and scales, modification of the legs, etc.

The case of *Titanoeca ingenua* (as well as that of *Titanoeca hesterna*) is still worse. The genus *Titanoeca* has been established by Thorell for spiders which belong to the division Cribellatæ. Scudder mentions the fact that this genus "is not far removed from *Amaurobius*," but in his description of both species

he fails to mention altogether either the calamistrum or the cribellum. In fact he does not mention even the spinnerets. Yet the spinnerets are visible in *Titanoeca ingenua* which is by far the better preserved species. The spinnerets are far apart, reminding of recent Drassids. No cribellum is visible. The legs are extraordinarily well preserved and the fourth metatarsi do not show anything like a calamistrum. It is therefore not probable that the spider in question is either a *Titanoeca* or any other cribellate species. But while the exclusion of this species from the division of Cribellatae is a comparatively simple matter, it is almost impossible to establish its true relationships. On account of its general appearance and the structure of the spinnerets I have placed both species provisionally under the family Drassidæ.

Of the forty-seven species of spiders presented in this paper, *Nephila pennatipes* and *Tetragnatha tertiaria* are the only ones whose generic identity seems to be sufficiently well established. The three species of *Thomisus* undoubtedly belong to the family Thomisidæ, but the genus in its modern conception remains uncertain. The same may be said about the species referred to the genus *Epeira* of the family Argiopidæ (Epeiridæ). The genera *Tethneus, Parattus* and several new genera proposed by me all deal with extinct forms and have therefore a different value from genera of recent spiders. None of the remaining spiders can be placed in the system satisfactorily. In these cases I did not feel justified to establish new generic names, a procedure which is advisable only where it is sure that the species does not belong to the genus under which it was originally placed. Each case will be discussed separately. In each such instance the genus used by modern arachnologists for recent species may have only the value of a vague approximation.

ORDER ARANEÆ.

Spiders represent a very natural order and already in the Upper Carboniferous or Pennsylvanic Period are easily distinguishable from all other Arachnida. In all recent spiders with the exception of the family Liphistiidæ of the sub-order Mesothelæ and in all Tertiary spiders known the abdomen appears unsegmented. In all spiders without a single exception the abdomen is joined to the cephalothorax by means of a very thin petiolus representing

the first abdominal somite. There is therefore a deep constriction between the carapace and the abdomen, whereas in Opiliones and Acari no constriction is noticeable because the abdomen is broadly joined to the cephalothorax. In Ricinulei the abdomen is also provided with a petiolus, but the coupling of the abdomen with the carapace is such that it has all appearance of a broadly joined abdomen. Moreover the Ricinulei possess a cucullus in front of the carapace. The Solifugæ have certain similarity with spiders although their abdomen is broadly joined to the carapace. What permits an easy separation of the Solifugæ from the spiders is the difference in the structure of their carapace and cheliceræ. The carapace in Solifugæ represents the fusion of the cephalic tergites with the first thoracic tergite, leaving the second, third and fourth thoracic tergites free, whereas in spiders all thoracic tergites are fused with the cephalic tergites. The cheliceræ of spiders are retrovert, those of Solifugæ chelate. From other orders of Arachnida in which the abdomen is also joined to the carapace by means of a petiolus the spiders can be distinguished by their cheliceræ and pedipalpi. In recent forms confusion is impossible. In extinct forms members of the sub-order Amblypygi of the order Pedipalpi could be easily confused with spiders if the preservation were deficient. In such cases the relative size and position of the coxæ comes to our aid. The possession of spinnerets on the abdomen is an exclusive characteristic of spiders, and if spinnerets are preserved spiders can be recognized as such even if all other characters are indistinguishable or missing.

Tertiary spiders possess already all the characters of recent spiders, including the epigynum in females and the copulatory apparatus on the terminal joint of the palpus in the male. The external appearance of Tertiary spiders is much the same as in common spiders of the present time and their habits, in a general way, must have been similar. It is difficult to say whether the paucity of material is attributable to the poor conditions prevailing in the Tertiary for the preservation of spiders, or to the relative paucity of the spider fauna itself of that period. For the recent spider fauna runs into several hundred as against the forty-seven Tertiary spiders of the same geographical region. Considering the numerous species preserved in the European amber we may reasonably assume that the real number of American Tertiary spiders must have been considerable.

SUB-ORDER MYGALOMORPHÆ.

Spiders with an unsegmented abdomen and two pairs of lungs. Articulation of cheliceræ such that the fangs move in a plane parallel to the plane of symmetry.

This sub-order comprises two families, Avicullariidæ and Atypidæ.

FAMILY AVICULARIIDÆ.

Usually four, sometimes two spinnerets. (Anterior spinnerets belonging to the fourth abdominal somite and found in Atypidæ and other spiders are missing in Avicullariidæ.) Eyes eight or six, usually situated on an elevation and forming a compact group. Anterior median eyes alone of the diurnal type. Coxæ of pedipalps without maxillary lobes.

A very large family comprising almost all recent Theraphosid spiders and practically limited to the equatorial and tropical belts. In this country the family is represented by some thirty species, most of which are distributed through the southern states and California. One species was found in Virginia, another in the Indian Territory. I have a single specimen of a species new to science and as yet unpublished from Indiana.

The separation into sub-families and genera of spiders belonging to the family Avicullariidæ is based on the following characters: structure of the tarsi and number of claws, number and structure of spinnerets, presence or absence of a rastellum on the cheliceræ, number of eyes and configuration of eyegroup, shape of carapace and sternum.

Being unable to place the single species of Theraphosid spider from Florissant under any of the numerous recent genera, I propose the new genus *Eodiplurina* for Tertiary forms more or less related to the recent sub-family Diplurinæ.

Genus **Eodiplurina**, new.

Theraphosid spiders with two pectinated claws and two pairs of spinnerets of which the posterior pair is three jointed and much longer than the anterior pair. Genotype: *E. cockerelli.*

Eodiplurina cockerelli n. sp. (*Text figures 1 and 2*).

A very well preserved specimen of a female, on both pieces showing only the ventral surface. Total length including chelicerae 15 mm. Abdomen 7 mm. long. Outline of carapace not visible.

FIGURE 1.—*Eodiplurina cockerelli* n. sp. x 13.

Chelicerae heavy, their inner margin with a row of dark, distinct granules and a thick scopula. The fangs are not well preserved. Coxa of pedipalps without maxillary lobe. Lip about as long as wide. Sternum almost circular. Anterior coxae widely

separate, posterior ones contiguous. Abdomen oval, lighter in color, sparsely covered with thick, simple hair, with a darker spot in the middle of the genital area. On one side of the dark spot two faint impressions, one behind the other, possibly imprints of the lung-books. The spinnerets are well preserved, covered with simple hair, spinning tubes not discernible. First pair of spinnerets small and far apart. Second pair large, three-jointed, at least three times as long and considerably stouter than anterior spinnerets. Pedipalps with a pectinate claw. Legs and pedipalps covered with simple, thick hair and scattered spines which are rather weak. Tarsal joints of legs apparently without scopula, with a pair of pectinate claws. The claws of the third right

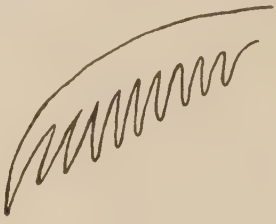

FIGURE 2.—*Eodiplurina cockerelli* n. sp. A claw of the third left foot. x 135.

tarsus (in the specimen on the left side) are especially well preserved. Its structure is shown in *textfigure 2* drawn with the aid of an Abbe drawing apparatus at a magnification of 135 diameters. The claws are with a series of from 8 to 9 teeth increasing in size from the base of the claw distally. Legs: 4123.

MEASUREMENTS OF THE LEGS IN MILLIMETERS.

	Femur	Patella + Tibia	Metatarsus	Tarsus	Total
I Leg	3.7	5.2	2.5	2.0	13.4
II Leg	3.6	?	?	?	13.0
III Leg	3.7	?	?	?	11.6
IV Leg	4.0	?	?	?	16.7
Pedipalp	2.3	3.1	2.0		6.4

The specimen is in the collection of Professor T. D. A. Cockerell of the University of Colorado and is from Florissant.

I am unable to place this species in any recent genus. It shows certain relationship with Brachythele which however have claws with a double series of teeth and other characters partly different partly indiscernible in *Eodiplurina cockerelli.*

Sub-Order Arachnomorphæ.

Spiders with an unsegmented abdomen and a single pair of lungs. Usually a single median tracheal spiracle in front of the spinnerets, sometimes a pair of them. Articulation of cheliceræ such that the fangs move in a plane intersecting the plane of symmetry.

This sub-order can be naturally divided into two divisions:

1 Division Cribellatæ.

True spiders with a cribellum in front of the spinnerets and a calamistrum on the fourth metatarsi.

Eight recent families belong to this division. Some of the species are exceedingly common. There are however no representatives of Cribellated spiders among the Tertiary spiders of North America.

2 Division Ecribellatæ.

True spiders without a cribellum or calamistrum. This division comprises 28 families of recent spiders. We shall mention only those families with which the Tertiary spiders are more or less directly concerned.

1 Sub-Division Haplogynæ.

This sub-division comprises six families of spiders in which the external genital organs are comparatively simple in structure and resemble those in Theraphosid spiders. Three of the six families, namely Oonopidæ, Dysderidæ and Caponiidæ, have two pairs of spiracles on the abdomen. In the Caponiidæ both pairs lead into tracheal tubes. In the Oonopidæ and Dysderidæ the first pair leads into lung-books, the second into tracheal tubes. In the remaining three families, namely Sicariidæ, Leptonetidæ and Hadrotarsidæ the spiracles are distributed so that a pair of them belongs to the lung-books, and a single median spiracle leads into the tracheal tubes.

The Caponiidæ have eight eyes, the Oonopidæ and Dysderidæ have six eyes. The Dysderidæ have a colulus in front of the spinnerets, the Oonopidæ have none.

Family Dysderidæ.

Characters of the family:—Two pairs of spiracles, the first of which belongs to lung-books, the second to tracheal tubes. Six eyes. Cheliceræ long and obliquely inclined. Lip very long. Maxillary lobes parallel. Anterior coxæ long, cylindrical Six spinnerets, anterior and posterior ones of about the same length while the median ones are sometimes shorter. Colulus present. Tarsi with two or three claws, upper claws with a single series of teeth, median claw smooth.

The sub-family Segestriinæ is characterized by the shape of the sternum which is much as in other spiders. The two genera, *Segestria* and *Ariadna,* of which the sub-family is composed, are separable by the position of their eyes and the structure of cheliceræ. Both genera are widely distributed.

Genus *Segestria* Latreille.

Segestria secessa Scudder (*Textfigure 3*).

Scudder, Tertiary Insects, 1890. P. 61. Pl. 11. fig. 28.

Two females, one of these being No. 205 (now No. 71) of the Scudder Collection of the Museum of Comparative Zoology of Harvard University, the other Nos. 1806 and 1818 of the Princeton Collection. Both from Florissant.

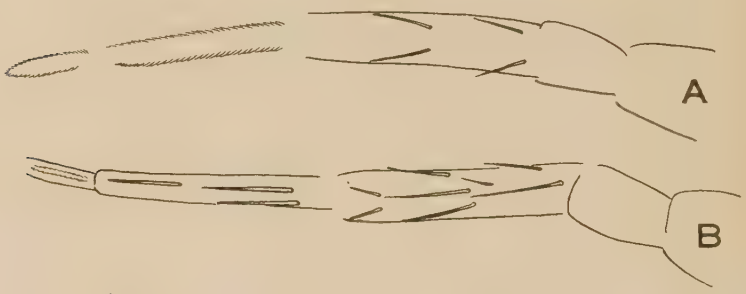

Figure 3.—*Segestria secessa* Scudder. Specimen No. 205 (71). Legs: a, first left; b, fourth left. x 13.

The Harvard specimen is the type of the species and the only one which I have examined. It is a beautifully preserved specimen although the impression is rather faint. The spiracles are altogether indiscernible. The claws are too poorly preserved to

be studied under higher power and the sternum is not clear. The generic affiliation of the species is therefore entirely based on external similarity with recent representatives of the genus Segestria. Scudder's description and measurements are correct and need not be repeated here. It may be added that there are two pairs of spines on the underside of the first, second and third tibia (the first left leg is shown in *textfigure 3a*). The metatarsi of the third pair have also two pairs of spines below. The tarsi and metatarsi of the first and second pair of legs show a distinct scopula of short hair. The fourth pair of legs has more spines than any other, but the spines are not so regularly arranged (*textfigure 3b*). Legs in order 4123.

FIGURE 4.—*Segestria scudderi* n. sp. × 3.33.

Segestria scudderi n. sp. (*Textfigure 4*).

A single female No. 16379 (now No. 117) of the Scudder Collection of the Museum of Comparative Zoology in Harvard University.

The specimen is almost complete, but poorly preserved, the surface of the rock being eroded and uneven. The specimen presents only the ventral surface. It may be separated from the preceding species by its somewhat larger size and the greater length of the fourth pair of legs as compared with the first pair. Body and legs are covered with long, simple hair. There are many spines on all legs, apparently forming two ventral rows of three spines each on all tibiæ and metatarsi, but the unevenness of the surface makes a definite counting impossible. The spiracles are not preserved, the spinnerets appear as a darker spot at the end of the abdomen, but their structure or number cannot be made out. The sternum is distinctly oval, longer than wide, but its margin is not clear. The cheliceræ are strong, their fangs are not visible. The pedipalpi are both well preserved, slender and short. The legs are rather robust, in the order 4123. The total length including cheliceræ is 11 mm. The carapace, not showing any outline of its margin, cannot be measured. The abdomen is 6.5 mm. long and 3 mm. wide and has more or less parallel sides.

Measurements of the Legs in Millimeters.

	Femur	Patella + Tibia	Metatarsus	Tarsus	Total
I Leg	3.2	3.1	1.6	1.4	9.3
II Leg	3.0	3.1	1.5	1.4	9.0
III Leg	3.0	2.4	1.5	1.5	8.4
IV Leg	3.6	3.7	2.3	1.8	11.4

I have placed this species represented by a single specimen from Florissant under the genus *Segestria* merely because of its external similarity with the preceding species.

2 Sub-Division Entelegynæ.

This sub-division comprises twenty-two families of spiders in which the external genital organs are more complicated. In the female the genital opening is provided either with a *claustrum* or an epigynum. In the male the copulatory apparatus on the terminal joint of the pedipalp is provided with variously formed chitinized structures in addition to the embolus.

To the families recognized by Simon, only few of which we shall consider here, I thought it advisable to add a new family Parattidæ for the four species of Tertiary spiders referred to the genus *Parattus*.

FAMILY DRASSIDÆ.

Characters of the family:—Eyes eight, heterogeneous, forming two rows of four eyes each. Anterior median eyes alone diurnal. Maxillary lobes with a distinct obliquely transverse depression. Tarsi with two claws and ungual tufts. Anterior spinnerets far apart. Colulus wanting.

Genus **Palæodrassus,** new.

With the characters of the family. The appearance of the spinnerets makes it fairly certain that these spiders belong to the same family with recent Drassids, but it would be impossible either to place them under any recent genus or to separate the genus *Palæodrassus* from recent genera. It is very likely that in reality the species described under this genus belong to several genera. Four of the species may be readily separated from each other by the order of their legs which is 4123 in *P. ingenuus,* 4213 in *P. interitus* and *P. hesternus* and 4321 in *P. cockerelli.* The order of legs in *P. florissanti* is not known. Genotype: *P. ingenuus.*

Palæodrassus ingenuus (Scudder) (*Textfigures 5, 6*).

= *Titanoeca ingenua* Scudder, Tertiary Insects, 1890. P. 69. Pl. 11, figs. 29 and 32.

Four females from Florissant in the Scudder collection of the Museum of Comparative Zoology of Harvard University, numbered 9792, 11203, 13520, 14031 are mentioned in Scudder's work. Of these specimens however numbers 11203 (now No. 82) and 13520 (now No. 83) are the obverse and reverse of the same specimen, while No. 9792 (now No. 81) seems to belong to a different species, yet is too poorly preserved to warrant separation. I have not seen specimen 14031.

Scudder's description of this species refers entirely to Nos. 11203 and 13520 and is correct. He made a mistake in placing it in the genus *Titanoeca* and in the family Agelenidæ to which that genus does not belong. I have already pointed out that the absence of a cribellum and calamistrum makes the retaining of this species in the genus *Titanoeca* impossible. Nor do I think that there is any likelihood of its belonging to the family Agel-

enidæ, representatives of which are easily recognized because of the relative length of their posterior spinnerets. Nor can it be a Clubionid because its anterior spinnerets are not close together, but far apart.

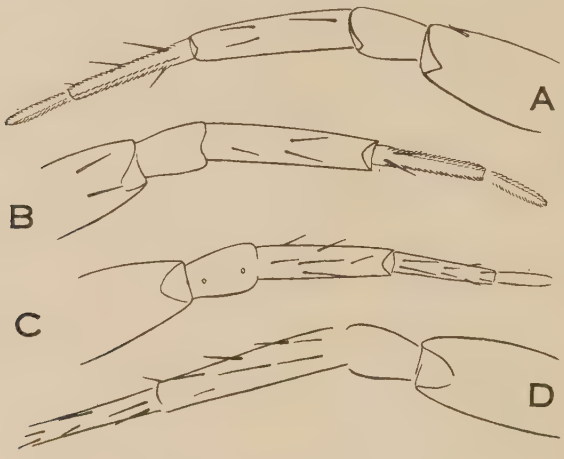

FIGURE 5.—*Palæodrassus ingenuus* (Scudder). Specimen No. 13520 (83). Legs: a, first left; b, second right; c, third right; d, fourth left. x 10.

The legs of this species are represented in our *textfigure 5*, the first and fourth being left legs, while the second and third right legs. It will be noticed that there is a distinct scopula on the first and second tarsi and metatarsi, a condition quite common among Drassids. There are three distinct spines on the underside of the first and second tibia, one of these close to the base, the other two forming a pair a little beyond the middle of the joint. There are also two or possibly three spines on the underside of the first and second metatarsi, and a pair of spines on the underside of the first and second femur close to the apical end of the joint. The spines on the third and fourth leg are more numerous. We find a median row of three equidistant spines on the underside of the tibiæ accompanied by several lateral spines, and three pairs of spines on the underside of the metatarsi apparently accompanied by a pair of lateral spines. There are also two circular sockets for spines on the third patella, but the spines themselves are missing as spines are often broken off in life and still more commonly after death. Legs in order 4123.

The specimen of *Palæodrassus ingenuus* presents also the peculiar spectacle of a well preserved epigynum (*Textfigure 6.*) Scudder has entirely overlooked its presence. It is surrounded by hair and its appearance is best understood from the drawing which I have made with the aid of an Abbe Drawing apparatus

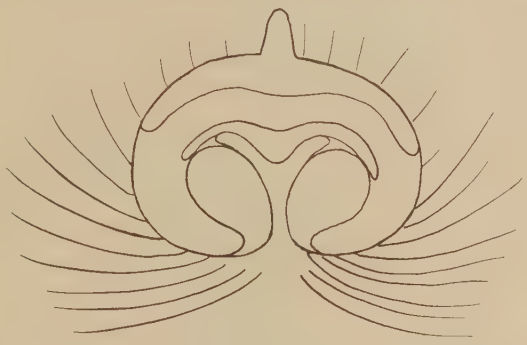

FIGURE 6.—*Palæodrassus ingenuus* (Scudder). Specimen No. 13520 (83). Epigynum. x 135.

at a magnification of 135 diameters. It reminds more that of a *Gnaphosa* rather than *Drassodes,* but in absence of any evidence derived from the study of the lower margin of the cheliceræ, which unfortunately is not distinct, the similarity in the epigynum would be insufficient to warrant the inclusion of the species in a definite genus.

Palæodrassus hesternus (Scudder) (*Textfigure 7*).

Titanoeca hesterna Scudder, Tertiary Insects, 1890, p. 69.

Four females, Nos. 5656, 12006, 12977 and No. 1809 of the Princeton Collection. Of these Nos. 5656 (now No. 84) and 12006 (now No. 85) are the only ones which I have had opportunity to examine and are both in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

The description given by Scudder is correct and I cannot add anything material to it, except a figure of specimen No. 5656 and the statement that the spinnerets are indiscernible and that there is no calamistrum on the fourth metatarsi. No. 12006 is less com-

plete but at the same time the better preserved of the two specimens. The hair and the heavy, long spines on the legs are quite clear, and the scopulæ on the first and second tarsi and metatarsi are also very distinct. Legs in order 4213.

FIGURE 7.—*Palæodrassus hesternus* (Scudder). Specimen No. 5656 (84). x 5.

Palæodrassus florissanti n. sp. (*Textfigure 8*).

One female from Florissant in the T. D. A. Cockerell Collection of the University of Colorado.

The specimen presents the ventral surface with the outline of the carapace superimposed. The specimen is very poorly preserved and is referred to this indefinite genus on account of its spinnerets. Of these the anterior ones are cylindrical, far apart, the posterior ones much shorter, approximated and occupying the space between the anterior ones. Total length of the spider including chelicerae—7 mm. The abdomen is somewhat pressed out of shape and is 4 mm. long. Sternum longer than wide, pointed posteriorly, truncated in front, more or less oval in shape. Lip longer than wide. Chelicerae weak, pedipalpi slender. The legs are not complete except for the right second leg which is 5.1 mm. long. The femora are heavy, widest in their distal third. Strong spines, some of black color, are on all femora and on the

preserved sections of tibiæ. There is also simple hair covering the entire body and legs. Nothing else can be made out.

FIGURE 8.—*Palæodrassus florissanti* n. sp. x 5.

Palæodrassus cockerelli n. sp. (*Textfigure 9*).

One female from Florissant in the T. D. A. Cockerell Collection of the University of Colorado. (Two pieces, both showing the ventral surface of the same specimen.)

Preservation very poor. Total length 8.5mm., abdomen 4.6mm. long. The legs of the right side (left on the specimen) are complete, and in order 4321. Total length of leg I—4.8 mm., leg II—5.1 mm., leg III—5.2 mm., leg IV—7.2 mm. Scopula present on first and second tarsi and metatarsi. Spines on third and fourth femur, tibia and metatarsus. Spinnerets visible, but their structure indistinct. Simple hair on body and legs. Pedipalps slender.

Palæodrassus interitus (Scudder) (*Textfigure 10*).

= *Anyphæna interita* Scudder, Tertiary Insects, 1890, p. 67. Plate 11, fig. 5.

One female from Florissant, Nos. 8269 (now 80) and 8281 in

FIGURE 9.—*Palæodrassus cockerelli* n. sp. x 5.

the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

That the spider in question does not belong to the genus *Anyphæna* under which Scudder had placed it "provisionally," induced to do so by "the general appearance of the specimen," cannot be doubted. In *Anyphæna* which is a Clubionid the anterior spinnerets are approximated and the tracheal spiracle is situated far in advance of the spinnerets. This spiracle may be seen in *Anyphæna* easier than in any other spider owing to the peculiar arrangement of the hair which forms two longitudinal lines parallel to each other and extending from the spiracle to the spinnerets. Scudder has not paid any attention to either of these characters. The spinnerets are represented in our figure 10 and are characterized by the wide separation of the anterior pair and the comparatively heavy appearance of the posterior pair. The median spinnerets are quite small. All spinnerets are covered with hair, but spinning tubes are not discernible. There is no trace of a spiracle but the arrangement of the hair on the abdomen does not

reveal anything in the shape of two parallel lines. The eyes cannot be made out clearly and I am unable therefore to verify Scudder's description of them. On the other hand the sternum,

FIGURE 10.—*Palæodrassus interitus* (Scudder). Specimen No. 8269 (80). Spinnerets. x 20.

not mentioned by Scudder, is clearly visible and has the shape of a very regular oval. Legs in order 4213. Other characters as well as the measurements given by Scudder are correct and there is nothing I could add to them.

FAMILY CLUBIONIDÆ.

Characters of the Family:—Eyes eight, in two rows of four eyes each, usually only the anterior median eyes of the diurnal type. Maxillary lobes without obliquely transverse depression. Tarsi with two claws and ungual tufts. Anterior spinnerets approximated. Colulus wanting.

So close is the relationship between this family and Drassidæ that for a long time the two were regarded as a single family. Simon in his great work has placed the Clubionidæ after the Thomisidæ with whom they certainly have a great deal of relationship, and before the Agelenidæ. He has enclosed in the family the Selenopidæ and the Ctenidæ. I think these two are better regarded as separate families. It seems to me further that the Salticidæ (Attidæ) show also distinct relationship with some Clubionids both in structure and in habits.

The separation of the Clubionidæ into sub-families is based on the structure of the maxillary lobes, of the lip and of the posterior spinnerets and on the position of the tracheal spiracle immediately in front of or considerably in advance of the spinnerets.

Scudder has described four species of *Clubiona* and one species of *Anyphæna*. The latter, as I have shown above, is a Drassid and not a Clubionid. Of the former I left only *Clubiona arcana* in the genus *Clubiona* on account of the order of the legs, which is the same as in recent species of that genus. For the other three species three new genera had to be established, based on the order of their legs. The number of species placed under the family Clubionidæ has been increased to seven by the addition of three new species.

Table for the Separation of Tertiary Genera of Clubionids.

1. The fourth leg is the longest.....Clubiona
   *Not so 2
2. The first leg is the shortest.....Eobumbratrix
   *Not so 3
3. Second leg as long as first .....Eoversatrix
   *Second leg much shorter.....Eostentatrix

Genus *Clubiona* Latreille.

The genus *Clubiona* as limited and defined at present is much more restricted than it was when Latreille first proposed it. It is difficult to say whether the three species referred by me to this genus really belong to it. It did not seem necessary to create a new genus although a number of recent Clubionids possess the same order of legs. As applied to Tertiary spiders we may define this genus as possessing the characters of the family and the legs in order 4123.

Clubiona arcana Scudder.

Scudder, Tertiary Insects, 1890, p. 64. Plate 11, fig. 4 (♂).

Scudder describes one male No. 2831 and three females Nos. 3253, 7087 and 8082 of the collection in the Museum of Comparative Zoology of Harvard University and one female No. 1. 807 and 1. 819 of the Princeton Collection. I have examined only No. 2831 (now No. 73), No. 3253 (now No. 74) and No. 8082 (now No. 75). The females are poorly preserved. Scudder's description is accurate. It may be added that the spinnerets which are visible in the male are approximated and that the male copulatory apparatus has unquestionable similarity to that of

recent Clubionids. Of course, the details of its structure cannot be made out nor can it be decided whether the females belong really to the same species with the male.

Clubiona florissanti n. sp. (*Textfigure 11*).

One female from Florissant in the collection of T. D. A. Cockerell of the University of Colorado.

FIGURE 11.—*Clubiona florissanti* n. sp. x 5.

The specimen is almost complete, but poorly preserved. It is even impossible to decide which surface is preserved, although the presence of spines in the median line of the legs makes one think that we have the dorsal surface before us. Total length 6.5 mm. Abdomen oval, 4.2 mm. long, 2.7 mm. wide in the middle. Measurements of legs:—First leg—6.1 mm., second—5.7 mm., third—5.0 mm. and fourth—6.8 mm. The individual joints are not clearly separable. Body and legs are clothed with simple

brown hair. It is difficult to decide whether the first and second tarsi have a scopula or not. There are many distinct spines on the legs, but their arrangement is not clear. The spinnerets are approximated, but displaced and poorly discernible. No other structures can be made out satisfactorily.

Clubiona curvispinosa n. sp. (*Textfigure 12*).

One female from Florissant, No. 16377 (now No. 120) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University. The specimen presents the ventral surface

FIGURE 12.—*Clubiona curvispinosa* n. sp. x 5.

and is complete, but rather poorly preserved. The legs of the fourth pair are superimposed over the abdomen, meeting far beyond its end. Total length of the spider—6.9 mm. Immediately behind the fourth coxæ which are contiguous, a darker area in the middle line apparently represents the epigynum. The spinnerets appear as a similar dark area at the end of the abdomen, but neither their shape nor their number can be made out. Tak-

ing these two areas as points for measurement we may assume that the abdomen is approximately 4.2 mm. long. The outline of the carapace is also visible, though indistinctly. The carapace seems to have been pressed out of shape, but in the live spider was probably very wide in front. The legs are in order 4123. The fourth leg is 7.6 mm. long, the first—6.3 mm. The interesting feature of the armature of the legs is furnished by the strong, curved spines on all femora. Less powerful but also curved spines are found on all other joints except the tarsi. The fourth tarsi alone are well preserved and are thickly clothed with hair. Entire body and legs are covered with both long and short, simple hair. No other structures are discernible.

Genus **Eoversatrix**, new.

With the characters of the family, but with the legs in order 1243. Genotype: *E. eversa.*

Eoversatrix eversa (Scudder).

= *Clubiona eversa* Scudder, Tertiary Insects, 1890, p. 63. Plate 11, fig. 22 (♂).

Scudder describes two males from Florissant, Nos. 5944 and 8551. Of these I have seen only the former which is now No. 72 of the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

Scudder's description is very good and need not be repeated here. All I have to add is that body, palpi and legs are clothed with simple hair. Also, Scudder does not mention the spinnerets which however are well distinguishable notwithstanding the fissure in the specimen. While one may not make out the details of their structure, it seems to be certain that they are all approximated. The order of legs 1243 is sufficient for the separation of this species in a separate genus, since in all recent species of *Clubiona* the fourth leg is the longest.

Genus **Eobumbratrix**, new.

With the characters of the family, but with the legs in order 2431. Genotype: *E. latebrosa.*

Eobumbratrix latebrosa (Scudder).

= *Clubiona latebrosa* Scudder, Tertiary Insects, 1890, p. 65 Plate 11, fig. 18 (♂).

One male from Florissant in the Scudder Collection of the Museum of Comparative Zoology of Harvard University, No. 6492 (now No. 76).

The description of Scudder is correct. The spinnerets are scarcely discernible, but seem to be approximated. The order of legs 2431 removes this species from all genera of recent Clubionids in which the third leg is always the shortest.

Genus **Eostentatrix,** new.

With the characters of the family, but with the legs in order 1423. Genotype: *E. ostentata.*

Eostentatrix ostentata (Scudder) (*Textfigure 13*).

= *Clubiona ostentata* Scudder, Tertiary Spiders, 1890, p. 65. Plate 11, fig. 24 (♂).

Two males Nos. 199 (now No. 77), 5507 and 5910 (now No. 78) and one female No. 9624 (now No. 79) from Florissant in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

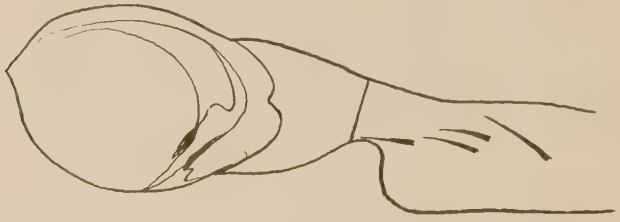

FIGURE 13.—*Eostentatrix ostentata* (Scudder). Specimen No. 5910 (78). Left pedipalp. x 40.

Scudder's description is correct. It may be added that the spinnerets are clearly visible in the male specimen No. 5910 and are approximated. The same specimen shows the pedipalps beautifully. The bulb of the copulatory apparatus has the appearance of recent Clubionids. The tibia of the pedipalp is distally

enlarged owing to the presence of an apophysis. The patella of the pedipalp has also an apophysis and is besides provided with four spines. The structure of the copulatory apparatus itself is not distinct, but appears as a dark area with curved edge possibly representing a long curved embolus. Legs in order 1423.

The female is very poorly preserved and it is impossible to decide whether she actually belongs to the same species.

Eostentatrix cockerelli n. sp. (*Textfigure 14*).

One female from Florissant in the collection of T. D. A. Cockerell of the University of Colorado.

The specimen is well preserved and shows the ventral surface with the outline of the carapace impressed over the coxæ. Total length 11 mm. Carapace 3.1 mm. long, 3.1 mm. wide, subrotund. Abdomen 5.5 mm. long, 4.8 mm. wide, joined to the carapace by a long petiolus. Cheliceræ week, slightly divergent. Pedipalpi slender and somewhat longer than carapace. Legs in order 1423. First leg—16.1 mm. long, second—15.0 mm., third—12.8 mm., fourth—15.9 mm. They are clothed with simple hair. Strong spines are present on all legs, but they are not numerous, nor can their arrangement be made out clearly. The spinnerets are approximated, the posterior ones longer than the anterior ones, clothed with simple hair, but spinning tubes indiscernible. The body is also covered with simple hair which is long and brown in color. Nothing else can be made out.

FAMILY PARATTIDÆ, NEW.

A family of extinct spiders of the sub-order Arachnomorphæ, division Ecribellatæ and sub-division Entelegynæ, having a general resemblance to Attids and jumping Clubionids and probably closely related to both. In absence of other tangible characters on account of the poor preservation of the specimens, the family may be characterized by the arrangement and proportion of the eyes, as described for *Parattus oculatus,* the main points being, that the eyes are round, in two rows of four eyes each, anterior eyes subequal and fairly equidistant, posterior eyes considerably smaller, the posterior median eyes situated between and slightly behind the anterior median eyes.

FIGURE 14.—*Eostentatrix cockerelli* n. sp. x 3.3.

Genus *Parattus* Scudder.

With the characters of the family.

Parattus resurrectus Scudder (*Textfigure 15*).

Scudder, Tertiary Insects, 1890, p. 53, Plate 11, fig. 26 (♀ ?).

One male No. 1081 (now No. 64) and one female Nos. 8459 and 8282 (now 65) from Florissant in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

I have discussed this case above and have little to add. Our *textfigure 15* shows the arrangement of the spines on all legs. As

the legs on the right side are completely preserved we can state positively that there is no scopula either on the tarsi or metatarsi. The claws are not well discernible, but it seems that there are only

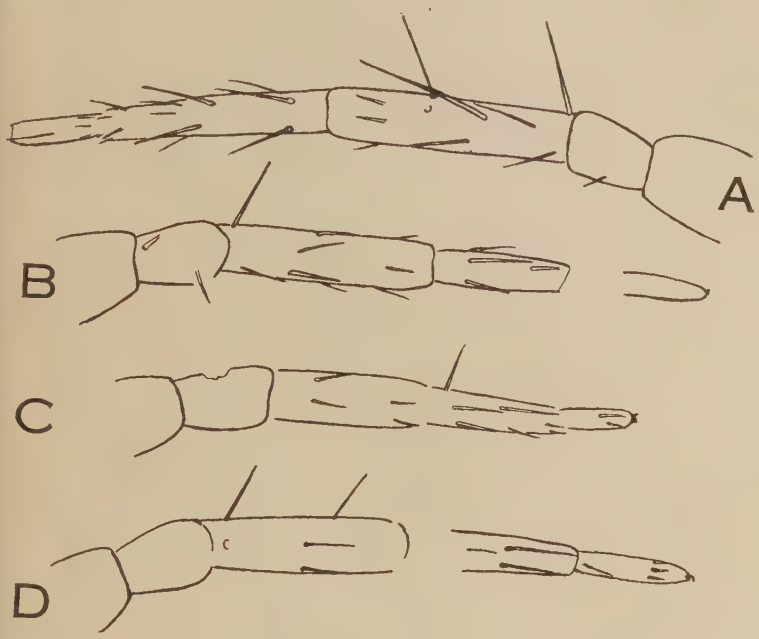

FIGURE 15.—*Parattus resurrectus* Scudder. Specimen No. 8282 (65). Legs: a, first left; b, second right; c, third right; d, fourth right. x 20.

two claws and that both are smooth. It would be impossible to decide whether the two specimens represent the male and the female of the same species. Legs in order 1243.

Parattus evocatus Scudder (*Textfigure 16*).

Scudder, Tertiary Insects, 1890, p. 54.

One female, No. 12005 (now No. 66) from Florissant in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

It is difficult to ascertain whether Scudder's description of the species is correct or erroneous. At one time I thought to see two rows of eyes, more or less equidistant and equal in size, one pro-

curved, the other recurved, but on reëxamination after the lapse of some time I was unable to verify my own rough sketch. Each femur shows a median row of three spines above and two or three

FIGURE 16.—*Parattus evocatus* Scudder. Specimen No. 12005 (66). x 5.

lateral spines. Body and legs are clothed with simple hair visible under higher power.

Parattus latitatus Scudder (*Textfigure 17*).

Scudder, Tertiary Insects, 1890, p. 55.

One specimen from Florissant, No. 9823 (now No. 67) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

The specimen is very poorly preserved and its sex as well as its generic affiliation remains uncertain. As far as can be ascertained, Scudder's description is correct. There are a few stout spines visible on the legs which are incomplete. The body is covered with simple hair.

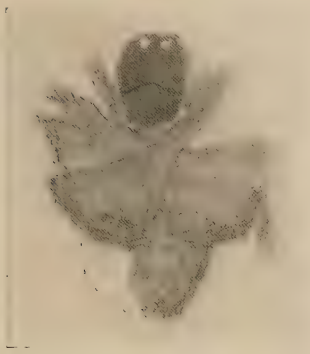

FIGURE 17. *Parattus latitatus* Scudder. Specimen No. 9823 (67). x 5.

Parattus oculatus n. sp. (*Textfigures 18 and 19*).

One female from Florissant, No. 118 of the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

FIGURE 18.—*Parattus oculatus* n. sp. Specimen No. 118. x 5.

An exceedingly poorly preserved specimen apparently presenting the dorsal aspect although there is no clear outline of the

carapace. Here and there a few spines may be seen on the legs, but even the lines between the joints cannot be made out. It is a small stout spider measuring 7 mm. in length. Order of legs 1243. Total length of leg I—8.0 mm., leg II—7.5 mm., leg III—6.8 mm., leg IV—7.2 mm.

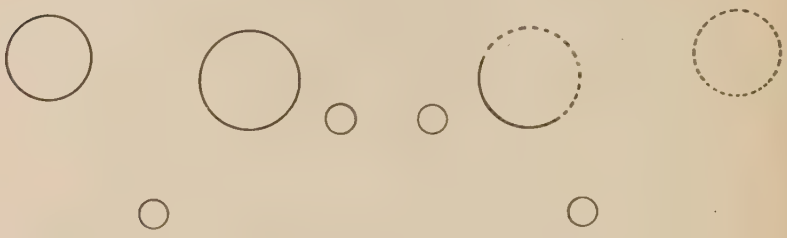

FIGURE 19.—*Parattus oculatus* n. sp. Specimen No. 118. Eyes. x 70.

The only really well preserved organs are the eyes (*textfigure 19*). They are eight in number and apparently only the right anterior lateral eye is completely indiscernible. Of the right anterior median eye a part of the outline is preserved and the remaining eyes are clearly visible. The anterior row is slightly procurved and much longer than the strongly recurved posterior row. The anterior median eyes are the largest and are about 1.75 their diameter apart. The anterior lateral eyes are also large, but distinctly smaller than the anterior median eyes and are separated from the latter by 1.50 their diameter. The eyes of the second row are considerably smaller, their diameter being about 3.50 times smaller than the diameter of the anterior median eyes. All eyes of the second row are of the same size. The posterior median eyes are situated between and slightly behind the anterior median eyes, are twice their diameter apart from each other and about one diameter from the respective anterior median eye. The posterior lateral eyes are behind and to the outside of the anterior median eyes from which they are removed by three diameters, while the distance between the posterior lateral and posterior median eyes is about six of their diameters. This arrangement of the eyes agrees closely with the description given by Scudder for *Parattus resurrectus* and is the reason why I have placed the otherwise so poorly preserved specimen without hesitation under the genus *Parattus*.

FAMILY THOMISIDÆ (LATERIGRADAE).

Characters of the Family:—Eight eyes in two rows, all diurnal, lateral eyes largest. Legs modified for lateral, crab-like locomotion. Tarsi with two claws, with ungual tufts. Spinnerets contiguous, with a distinct colulus in front of them.

Although several groups of spiders have laterigrade locomotion, the Thomisids are usually easily recognizable. Usually they are smaller than either the Selenopidæ or the Heteropodidæ and are distinguishable from the former by the position of the eyes and from the latter by the structure of the metatarsi. Moreover neither of these has a colulus. The generic distinction of Thomisids involves the study of the maxillary lobes, lip, sternum, margines of cheliceræ, hair etc., all characters not sufficiently apparent or completely indiscernible in extinct specimens. The three species which Scudder refers to the genus *Thomisus* are unquestionably Thomisids and his choice of genus is fortunate, for we may again regard it as merely a representative, indeterminate genus.

Genus *Thomisus* Walckenaer.

Thomisus resutus Scudder.

Scudder, Tertiary Insects, 1890, p. 57, Plate 11, fig. 13.

A single specimen of uncertain sex from Florissant, Nos. 5502 (now No. 68) and 7521 in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

The specimen is poorly preserved and its description given by Scudder is correct in all details.

Thomisus disjunctus Scudder.

Scudder, Tertiary Insects, 1890, p. 58, Plate 11, fig. 9.

Scudder describes two specimens from Florissant, one No. 9677 (now No. 69) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University, the other No. 10377 which I have not seen.

Scudder's description is correct except for his statement that "the tibiæ and first tarsal joint are completely consolidated into a single piece, so that the line of demarkation can not be seen." Examination of the legs under higher power reveals the pres-

ence of a semicircle of short hair, marking the end of the tibia and the beginning of the interarticulate membrane between the tibia and the metatarsus. Short hair is discernible on all joints of the legs.

Thomisus defossus Scudder (*Textfigure 20*).

Scudder, Tertiary Insects, 1890, p. 59, Plate 11, fig. 23 (♂).

One male from Florissant, No. 4742 (now No. 70) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

Scudder's description is correct and all I have to add is that I was able to expose the end of the third right tarsus and thus reveal the presence of two curved claws (*textfigure 20*). The left claw is somewhat turned sideways and partly covered with what seems to be hair but may be remnant of flat sharp-pointed ungual tufts. The right claw shows distinctly a single median tooth.

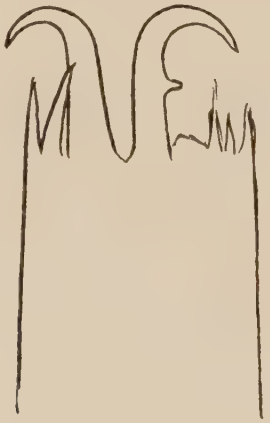

FIGURE 20—*Thomisus defossus* Scudder. Specimen No. 4742 (70). Claws of right third foot. x 135.

FAMILY LINYPHIIDÆ.

Characters of the Family:—Eight eyes in two rows, anterior median eyes alone diurnal. Spinnerets approximated, preceded by a colulus. Cheliceræ with oblique margins. A stridulating organ on the outer surface of the cheliceræ with a corresponding part on the inner surface of the pedipalpi. No comb on fourth tarsi. Clypeus high.

The separation into sub-families is based chiefly on the structure of the palpi in both sexes and the separation into genera on characters the majority of which are not easily ascertainable even in recent forms, especially in the case of females. Most of the recent spiders belonging to the sub-family Erigoninæ are minute, those of the sub-family Linyphiinæ larger and often resembling Theridiids with which they were originally classified, but from which they are easily separable by the structure of their fourth tarsi and cheliceræ.

Genus *Linyphia* Latreille.

Scudder has placed a single species under this genus. I have placed under it Scudder's *Theridium seclusum* which is certainly not a Theridiid and two new species. All these species have the general appearance of recent Linyphias, but it is impossible to say whether they actually belong to this genus.

Linyphia seclusa (Scudder).

= *Theridium seclusum* Scudder, Tertiary Insects, 1890, p. 74, Plate 11, fig. 20 (♂).

Scudder described three specimens, but one of these is a distinctly different species and has been placed by me under the genus *Palæopachygnatha*. This leaves two specimens of which No. 7816 I have not seen. The figure and description given by Scudder referr to specimen No. 9026 (now 88) of the Collection of the Museum of Comparative Zoology of Harvard University, which I have carefully examined.

It is a well preserved male. The description given by Scudder is correct. It must be added that the fourth tarsi are well preserved and show no trace of a comb. There are distinct spines of the Linyphiid type on all joints except tarsi.

Linyphia retensa Scudder.

Scudder, Tertiary Insects, p. 75, Plate 11, figs. 25, 27 (♂).

A single male and its reverse have been described by Scudder under the Nos. 12976, 13212 and 14032. Of these I have seen only No. 12976 (now No. 89) of the Scudder Collection of the Museum of Comparative Zoology of Harvard University. The

specimen is very poor and as far as I can judge Scudder's description is correct.

Linyphia florissanti n. sp. (*Textfigure 21*).

One specimen, probably a male, from Florissant in the T. D. A. Cockerell Collection of the University of Colorado. It is very poorly preserved.

I am not at all certain under which family this specimen should be placed. There are no spines on the legs which are clothed with simple hair. The claws are not preserved, the eyes are indiscernible, neither can the spinnerets be seen. Only its general appearance reminds of a Linyphiid.

FIGURE 21.—*Linyphia florissanti* n. sp. x 3.33.

Total length including chelicerae—6.2 mm. Carapace longer than wide, being 2.1 mm. long and 1.9 mm. wide in the widest place, i.e. between the second and third coxæ. It is eggshaped, pointed anteriorly. The outline of the sternum appears impressed inside the carapace. Its anterior end is not clearly visible, but the first coxæ are distinctly widely separate. The posterior end of the sternum is pointed, slightly yet distinctly separating the fourth

coxæ. The cheliceræ are large and heavy. A dark area not shown in the drawing, but having the appearance of a copulatory apparatus of a male pedipalp, is visible at a little distance in front of the cheliceræ. The abdomen is perfectly oval. The legs are long, in order 1243. The end of the first leg is difficult to trace, but the entire leg is considerably heavier than the fourth leg. Measured to its visible end it is 15.7 mm. long, the second leg—13 mm., the third—6 mm., and the fourth—9 mm.

Linyphia pachygnathoides n. sp. (*Textfigures 22 and 23*).

One male from Florissant, No. 38124 in the Collection of the U. S. National Museum.

FIGURE 22.—*Linyphia pachygnathoides* n. sp. Specimen No. 38124. × 3.33.

The name chosen for this species is to indicate a close resemblance in the structure of its pedipalp to that in the recent genus *Pachygnatha*. As our *textfigure 23* shows, the tibia of the pedipalp has no apophysis. The end of the tarso-metatarsal joint pro-

jects beyond the copulatory apparatus. The structure of the latter is not clearly discernible, yet it seems to have a more or less spiral arrangement as indicated by dark streaks represented as lines in the drawing.

FIGURE 23.—*Linyphia pachygnathoides* n. sp. Specimen No. 38124. Right pedipalp. x 70.

Total length of spider—about 4 mm. Abdomen—2.5 mm. long, 1.3 mm. wide. The legs are slender, in order 1243. They are covered with short brown hair. Here and there a few weak, short spines are visible, forming various angles with the axis of the leg (from 24 to 58 degrees). The separate joints are clearly visible only in the first pair of legs. Here the femur measures 3.7 mm., patella with tibia—4.5 mm., metatarsus with tarsus—6.8 mm., total 15.0 mm. In the second leg only the metatarsus is clearly separated from the tibia and together with the tarsus measures 5.0 mm., while the total length of this leg is 12 mm. The third pair of legs is poorly preserved and cannot be even correctly measured. As far as visible it measures about 5 mm. The fourth pair of legs is complete although separation of the joints is not clear. Its total length is 9 mm.

FAMILY ARGIOPIDÆ.

Characters of the Family:—Eight eyes in two rows, all diurnal. Spinnerets approximated, preceded by a colulus. Cheliceræ with oblique margins with several teeth. Legs with spines. Tarsi with three claws and with several spurious claws (serrated bristles).

The family is usually divided into three sub-families which may be distinguished as follows:

Chelicerae without boss at base.....Tetragnathinæ
Chelicerae with a boss at base
Lip longer than wide .....Nephilinæ
Lip wide and short.....Epeirinæ

SUB-FAMILY TETRAGNATHINÆ.

Genus **Palaeometa,** new.

Presumably with the characters of the sub-family, but with eyes on a transversely oval elevation. Genotype: *P. opertanea.*

Palaeometa opertanea (Scudder).

= *Theridium opertaneum* Scudder, Tertiary Insects, 1890, p. 73. Plate 11. fig. 3 (♀).

One female from Florissant, No. 13521 (now No. 86) in the Collection of the Museum of Comparative Zoology of Harvard University.

The specimen is imperfectly preserved and inadequately described by Scudder. Total length 11 mm. Carapace 5.0 mm. long, 2.2 mm. wide, abdomen 6.4 mm. long, almost globular. The legs are slender, imperfectly preserved. The third and fourth leg of the left side appear superimposed over the abdomen, the tip of the fourth leg protruding beyond the abdomen and at the first glance simulating a spinneret. However, it is not difficult to trace the leg on the surface of the abdomen owing to the arrangement of the hair which presents a different direction from that of the hair covering the abdomen, as the leg lies at an angle to the main axis of the spider. There are distinct spines on the first and second pair of legs, giving them an appearance entirely different from that of recent Theridiids. Moreover there is no trace of a comb on the fourth tarsus. This makes the retention of the species in the genus to which Scudder referred it impossible. The proportion of the femora indicates the probable proportion of the legs and is as given by Scudder: first femur—6.0 mm., second—5.0 mm., third—2.0 mm., fourth—3.25 mm.

The carapace shows a large transversely oval area which has the shape of a depression in the rock and which therefore represented an elevation in life. It seems probable that this represents the entire eyegroup, but the individual eyes cannot be made out. No other structures are discernible.

Genus **Palæopachygnatha,** new.

Spiders resembling recent *Pachygnatha.* Cheliceræ strongly divergent. Genotype: *P. scudderi.*

Palæopachygnatha scudderi n. sp. (*Textfigure 24*).

= *Theridium seclusum* Scudder ad partem specimen No. 2286 (now No. 87) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

FIGURE 24.—*Palæopachygnatha scudderi* n. sp. Specimen No. 2286 (87). × 3.33.

One specimen from Florissant, probably a female.

This species, which Scudder has erroneously identified with his *Theridium seclusum,* is at once easily separable from the latter by the difference in the relative length of its legs and the appearance of its cheliceræ. The cheliceræ are strongly divergent, a circumstance which decided me in placing the species under a genus allied to *Pachygnatha* with which the spider has external resemblance. The specimen has a very faint appearance, yet is well preserved. Its anterior legs as well as the legs of the second pair are broken off across the femora. The third and fourth pair are complete, but the claws are indiscernible. There is no trace of a comb on the fourth tarsi. The legs are clothed with simple brown hair and show a few spines. One of the spines is plainly

visible on the third right tibia, two spines on the fourth left metatarsus.

Total length with cheliceræ 7 mm. Carapace oval, apparently slightly longer than wide, although its anterior edge is not clearly defined. The abdomen is oval. The total length of the third leg is 7.1 mm., that of the fourth—11.6 mm. The pedipalpi are not discernible.

Palæopachygnatha cockerelli n. sp. (*Textfigure 25*).

One male from Florissant in the T. D. A. Cockerell Collection of the University of Colorado.

FIGURE 25.—*Palæopachygnatha cockerelli* n. sp. × 3.33.

The specimen, presenting the dorsal surface only, is not very well preserved and little detail can be made out. In general the spider has the appearance of a recent *Pachygnatha*. The cheliceræ are hidden under the copulatory apparatus of the pedipalpi,

but are apparently divergent. The carapace is slightly longer than wide. The abdomen is oval, widest in its middle, evenly narrowed at both ends. Legs in order 1243. They are clothed with simple brown hair. There are distinct spines on all legs, especially on the femora.

Total length of spider about 6.8 mm. The first leg measures 13.5 mm., the second—10.5 mm., the third—4.5 mm., and the fourth—9 mm.

Genus *Tetragnatha* Latreille.

Tetragnatha tertiaria Scudder (*Textfigure 26*).

Scudder, Arachnida in Zittel's Handbuch der Paleontologie, Vol. I, 11, p. 744, Fig. 927, 1885.

Scudder, Tertiary Insects, 1890, p. 77, Plate 11, fig. 11 (♂).

One male from Florissant, Nos. 5000 (now No. 62) and 5898 (now No. 90) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

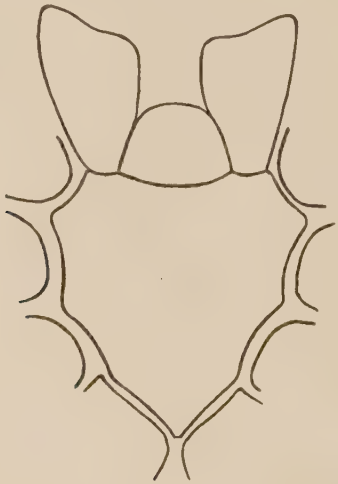

FIGURE 26.—*Tetragnatha tertiana* Scudder. Specimen No. 5898 (90). Sternum, lip and maxillary lobes. x 13.

A very well preserved specimen. Scudder's description is not complete omitting to mention such structures as the lip, maxillary lobes, sternum and spinnerets. Moreover he mistook the maxillary lobes for what he terms "the basal piece of the mandibles."

The sternum, represented in our *textfigure 26,* is wide in front, pointed behind, scarcely separating the hind coxæ. It is slightly emarginate anteriorly, receiving the wide underlip at the sides of which the maxillary lobes may be seen. They are more or less typical of *Tetragnatha* being considerably wider at the end than at the base and in a general way parallel to each other.

In front of the maxillary lobes the cheliceræ may be plainly seen. They have been probably pressed out of their position as their base is now in contact with the anterior edge of the maxillary lobes, a circumstance which misled Scudder in his interpretation of these structures. The cheliceræ are comparatively short and stout and strongly divergent. The left shows the fang complete. It is a little shorter than the basal joint, lighter in color, smooth and slightly but evenly curved. Considering that the specimen is unquestionably a male the cheliceræ are not typical, or perhaps I should better say not of the extreme type found in some recent species of the genus, but more of the type of the recent female *Tetragnatha laboriosa.* Impressions of the copulatory apparatus of the pedipalpi may be seen at a little distance in front of the cheliceræ, but the other joints of the pedipalp, which probably was slender, are not discernible.

The carapace is clearly visible on the specimen No. 5000 (now No. 62). Its anterior edge extends a little beyond the base of the cheliceræ, cutting them across. I have mentioned that the cheliceræ are apparently dislocated. The median line of the carapace comes to lie not between the chaliceræ, but slightly to the left, so that of the eyes which are plainly impressed across the base of the cheliceræ the right median eye lies in the juncture line of the two cheliceræ. The carapace is anteriorly narrower than posteriorly. Its widest place is between the second and third pair of coxæ where it measures 2.6 mm. while the length of the carapace is 3.5 mm. It is therefore distinctly longer than wide and oval in shape. Between the posterior edge of the carapace and the anterior edge of the abdomen a narrow space is bridged by the petiolus which has the shape of an elongated pentagon with the apex reaching the sternum and with sides slightly concave. Measured without the petiolus the abdomen is 4.6 mm. long and 2.3 mm. wide. The total length of the spider including chelicera is 10 mm.

The two rows of eyes are very slightly recurved and the posterior row slightly longer than the anterior row. All eyes are round

and subequal in size, only the anterior median ones slightly but distinctly smaller than the others. The eyes of the anterior row are separated from each other by equal distances measuring less than their diameter, but more than their radius. The same is true of the posterior row, the greater length of this row being due to the equal size of its eyes and to the ever so slightly greater distance between them. The rows are to all purposes parallel and the free distance between the rows is twice the diameter of the eyes. The quadrangle of the median eyes is therefore considerably longer than wide. The clypeus is unusually narrow, measuring less than the diameter of the anterior middle eyes. It may be added that the eyes of the anterior row are quite plainly visible, while those of the posterior row are discernible only when the light falls under a certain angle.

The spinnerets are well preserved on specimen No. 5898 (now No. 90). The anterior pair is almost contiguous. They have the appearance of brown discs with a smaller and well circumscribed disc inside the larger. This represents probably the terminal article of the spinneret, but no spinning tubes are discernible. The posterior spinnerets are smaller and are separated from each other by at least their diameter. Between the posterior spinnerets there is a darker area possibly representing the median spinnerets. In front of the spinnerets the colulus can be plainly seen as a small brown disc.

The first and second pair of legs are broken off apparently across the tibiæ. The third and fourth pair are complete. Scudder did not see the ends of their tarsi. When fully exposed the third leg measures 9.8 mm., the fourth—19.2 mm. The length of the femora is given correctly by Scudder as follows:—First femur 8.75 mm., second—7 mm., third—3.5 mm., fourth—7 mm. One can see median and lateral spines on all femora and tibiæ and some spines on fourth metatarsi. The hair is very faintly visible on legs, but is indiscernible elsewhere.

SUB-FAMILY NEPHILINÆ.

Genus *Nephila* Leach.

Nephila pennatipes Scudder.

Scudder, Arachnida in Zittel's Handbuch der Paleontologie, 1885, Vol. I, ii, p. 744, fig. 926.

Scudder, Tertiary Insects, 1890, p. 89, Plate 11, fig. 12.

One female, No. 11651 (now No. 61) from Florissant in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

Scudder's description is entirely correct in every detail.

SUB-FAMILY EPEIRINÆ.

This is a very large sub-family and the separation into groups and genera is based on many characters, some of which may be expected to remain preserved even in paleontological material, while others, like the difference in the structure of the inner and outer claws, certainly would not be discernible. Scudder places under the genus *Epeira* six distinct species, one specimen which is only partly preserved and several pieces with nothing but a leg on each. He also refers four species to a genus *Tethneus* established by him for these species. The history of the genus *Epeira* and its prototype *Aranea* shows how difficult it is to find permanent characters by which to distinguish the spiders referred to *Epeira* and a dozen or so of related genera. I consider it a mistaken attitude to abolish the genus *Tethneus* which after all is not applicable to recent spiders, and shall quote here the definition given by Scudder.

Genus *Tethneus* Scudder.

Spiders "compact in form, with short and stout legs of not very unequal length, and in particular the first two pairs of legs are unusually heavy. The second and fourth pairs of legs are of nearly equal length, or the second pair may be slightly longer; the femora of the first and second pairs of legs are at base as broad as or even broader than half the width of the cephalo thorax, and the longest legs are less, generally considerably less, than twice as long as the body" (p. 78).

Besides the four species described by Scudder I refer to this genus two new species.

Tethneus guyoti Scudder (*Textfigure 27*).

Scudder, Tertiary Insects, 1890, p. 78, Plate 11, figs. 8 (♂), 10 (♀).

Scudder described one female, No. 320 (now No. 91) and one

male, Nos. 8265 (now No. 92) and 8311 in the Collection of the Museum of Comparative Zoology of Harvard University and one male, Nos. 1. 808 and 1. 854 in the Princeton Collection. I have examined only Nos. 320 and 8265.

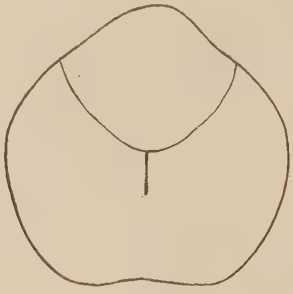

FIGURE 27.—*Tethneus guyoti* Scudder. Specimen No. 8265 (92). Carapace. x 13.

Scudder's description of the carapace is not quite correct. It is much narrowed in front (*textfigure 27*). The cephalic area is clearly separated by the cephalothoracic groove. A longitudinal groove begins at the cephalothoracic groove and runs backwards for a little over one-third of the length of the thoracic area. Neither eyes nor claws can be made out. The legs are covered with brown hair. Here and there one can see spines, but these are poorly preserved and barely discernible. The female undoubtedly presents the ventral aspect, but is so poorly preserved that nothing but the legs can be seen more or less clearly. Under such circumstances it is absolutely impossible to say whether the female belongs to the same species with the male.

The measurements given by Scudder are correct.

Tethneus obduratus Scudder.

Scudder, Tertiary Insects, 1890, p. 79, fig. 31 (♀).

One female from Florissant, No. 7177 (now No. 93) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

This specimen is very poorly preserved and the description given by Scudder, as far as can be ascertained, is correct.

Tethneus hentzii Scudder (*Textfigures 28 and 29*).

Scudder, Arachnida in Zittel's Handbuch der Paleontologie, 1885, Vol. I, ii, p. 744, fig. 928.

Scudder, Tertiary Insects, 1890, p. 80, Plate 11, fig. 14 (♂).

Scudder describes seven specimens, all males, Nos. 1226 (now No. 63), 1447 (now No. 94), 3860 (now No. 95), 6600 (now No. 96), 8635 (now No. 98) and its reverse 8533 (now No. 97), 14982 (now No. 99) and 8689, all in the Collection of the Museum of Comparative Zoology of Harvard University. Of these

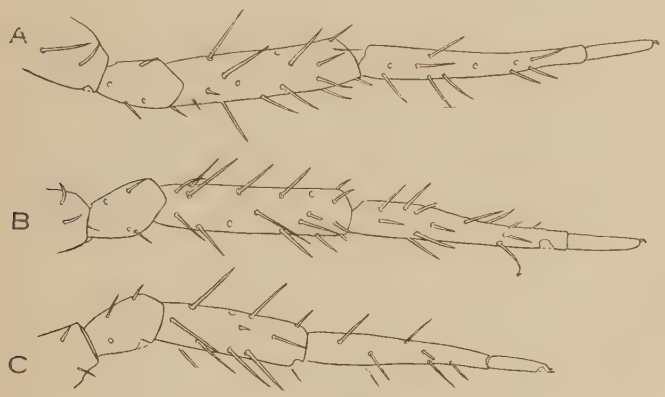

FIGURE 28.—*Tethneus hentzii* Scudder. Specimen No. 8635 (98). Legs: a, first right; b, second right; c, third right. x 10.

I have seen only specimen No. 8689. Scudder's description and measurements are correct. The best preserved specimen is that which is now numbered 97 and 98. Our *textfigure 28* shows the arrangement of spines on the first, second and third legs. The fourth leg does not possess as many spines, but as it is less perfectly preserved I refrain from giving a drawing of it. The legs are complete and show the claws. Only one claw however is really perfectly preserved. It is an upper claw of the first left leg of specimen No. 98 and is reproduced in our *textfigure 29* at a magnification of 440 diameters as drawn with the aid of an Abbe drawing apparatus.

Scudder's description of the species and his measurements are correct and I have nothing more to add to them.

FIGURE 29.—*Tethneus hentzii* Scudder. Specimen No. 8635 (98). A claw of the first left foot. x 440.

Tethneus provectus Scudder (*Textfigure 30*).

Scudder, Tertiary Insects, 1890, p. 81, Plate 11, fig. 21 (♀).

One male and three females from Florissant, Nos. 8141 (now No. 100), 13519 (now No. 101) and its reverse 13522 (now No. 102), 14991 (now No. 103) and 13524, all in the Collection of the Museum of Comparative Zoology of Harvard University. The last mentioned specimen I have not seen.

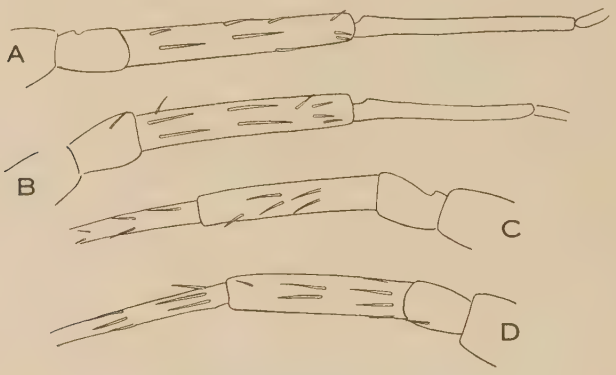

FIGURE 30.—*Tethneus provectus* Scudder. Specimen No. 14991 (103). Legs: a, first right; b, second right; c, third left; d, fourth left. x 10.

It is difficult to say whether all specimens belong to the same species. The arrangement of the spines on the legs of specimen No. 103 is shown in our *textfigure 30*. This being a male, a comparison with the preceding species is in order, and shows that *T. provectus* has not nearly as many spines as *T. hentzii,* but that

its spines have a more regular arrangement. Mention must be made of the presence of hair in the area behind what Scudder described as the abdomen in specimen No. 13522. The hair covered area extends all the way to the end of the hind legs. If this area represents the actual end of the abdomen, as is very likely, then the abdomen is much longer than it is represented in Scudder's description. In every other respect his description is correct.

Tethneus twenhofeli n. sp. (*Textfigure 31*).

One male in the collection of W. H. Twenhofel of the University of Kansas.

FIGURE 31.—*Tethneus twenhofeli* n. sp. x 3.33.

The specimen is fairly well preserved and presents the dorsal surface with coxæ and cheliceræ impressed on the carapace. Pedipalpi and legs are complete except for those of the fourth pair one of which is broken off across the tibia and the other across the metatarsus. The spider has a close resemblance to some recent *Epeira,* but I placed it under *Tethneus* on account

of the greater size of its first and second femora as compared with those of the third and fourth leg. The abdomen is broken off near its tip and the total length of the spider may be therefore given only approximately as 7.8 mm. The carapace is longer than wide, narrower in front, widest in posterior third, with a distinct longitudinal groove. The eyes are indiscernible. An interesting feature of the two anterior pairs of legs is the considerable difference in the diameter of the end of the tibia and the base of the metatarsus. There can be no doubt that the fourth leg was longer than the third and the legs are therefore in order 1243.

Measurements of legs: First leg, femur—4 mm., tibia with patella—4 mm., metatarsus with tarsus—5 mm., total 13 mm. Second leg, femur 3.4 mm., tibia with patella—3.8 mm., metatarsus with tarsus—3.8 mm., total—11 mm. Third leg—femur—2.5 m., tibia with patella—2.5 mm., metatarsus with tarsus—2.5 mm., total—7.5 mm. Fourth leg, femur—2.6 mm., tibia with patella—2.5 mm.

There are numerous heavy spines on all legs, and legs and body are clothed with short, simple, brown hair.

The pedipalpi show a large copulatory apparatus the details of which cannot be made out but the outward appearance of which reminds that of recent Epeiridæ.

Tethneus robustus n. sp. (*Textfigure 32*).

One male from Florissant, No. 16412 (119) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

The specimen presents the dorsal surface, shows the characteristic difference in the size of the femora, but is on the whole rather poorly preserved.

Total length 6 mm. Carapace oval, slightly narrower in front than behind, 2.4 mm., long, 1.8 mm. wide. The four median eyes alone are preserved. They are round, equal in size and the quadrangle is almost square. Legs in order 1243, those of the first and second pair much heavier and provided with numerous spines. There is also simple brown hair on body and legs. The pedipalpi are rather unusually well preserved although the details naturally cannot be made out. Nevertheless one may discern in the right

copulatory apparatus a deeply forked organ which probably represents both the embolus and conductor.

FIGURE 32.—*Tethneus robustus* n. sp. Specimen No. 16412 (119). x 5.

Measurements of legs: First leg, femur—2.7 mm., tibia with patella—3.2 mm., metatarsus with tarsus—3.9 mm., total 9.8 mm. Second leg, femur—2.5 mm., tibia with patella—2.7 mm., metatarsus with tarsus—3.1 mm., total—8.3 mm. Third leg, femur—1.4 mm., tibia with patella—1.6 mm., metatarsus with tarsus—2.0 mm., total—5.0 mm. Fourth leg, total—7.0 mm.

Genus *Epeira* Walckenaer.

Scudder has described six distinct species from Florissant and I have added two new species. All these species are undoubtedly close to recent spiders grouped under the cohors Araneus which notwithstanding the splendid efforts of the late Frederick P.

Cambridge still defies the ingenuity of arachnologists who want to split it into a series of genera.

Epeira meekei Scudder.

Scudder, Tertiary Insects, 1890, p. 83, Plate 11, figs. 2 (♀), 17 (♂).

Two males, Nos. 9211 (now No. 104) and 8221, and one female No. 3204 (now No. 105) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University. Scudder's description is correct.

Epeira abscondita Scudder.

Scudder, Tertiary Insects, 1890, p. 84, Plate 11, fig. 7 (♂).

One male from Florissant, No. 7583 (now No. 106) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

Scudder's description is correct, but his measurements are incomplete. I have exposed all legs which are in order 1243. First leg, femur—2.8 mm., tibia with patella—3.4 mm., metatarsus with tarsus—4.3 mm., total—10.5 mm.; Second leg, femur—2.9 mm., tibia with patella—3.0 mm., metatarsus with tarsus—4.3 mm., total—10.2 mm.; Third leg, femur—2.6 mm., tibia with patella—1.8 mm., metatarsus with tarsus—2.3 mm., total—6.7 mm.; Fourth leg, femur—3.4 mm., tibia with patella—2.3 mm., metatarsus with tarsus (broken off very near its distal end)—3.1 mm., total—8.8 mm.

Epeira delita Scudder.

Scudder, Tertiary Insects, 1890, p. 85, Plate 11, fig. 6 (♂?).

One specimen from Florissant, No. 13523 (now No. 107) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

Scudder's description is correct. The pedipalpi are absent, but the shape of the body and the proportion of the legs leave little doubt as to the sex of the specimen.

Epeira cinefacta Scudder.

Scudder, Tertiary Insects, 1890, p. 85, Plate 11, fig. 16 (♂).

One male from Florissant, Nos. 8576 (now No. 108) and 8806 (now No. 109).

Scudder's description is correct. The appearance of the specimen reminds one rather of recent *Mangora* than *Epeira*.

Epeira vulcanalis Scudder (*Textfigure 33*).

Scudder, Tertiary Insects, 1890, p. 86.

One male from Florissant, No. 5784 (now No. 110) in the Collection of the Museum of Comparative Zoology of Harvard University.

FIGURE 33.—*Epeira vulcanalis* Scudder. Specimen No. 5784 (110). × 5.

Scudder's description is correct. As he omitted to give a figure of the spider it seemed to be advisable to give one now. It may be added that the spinnerets appear as a dark brown disc a little in advance of the posterior end of the abdomen. An interesting feature is the dark line surrounding the disc and reminding one of the circular wall around the spinnerets in recent Gasteracantha.

Epeira emertoni Scudder.

Scudder Tertiary Insects 1890, p. 87, Plate 11, figs. 15 (♂), 19 (♀).

One male, No. 8777 (now No. 111) and two females, Nos. 10998 (now No. 112) and 5117. The latter I have not seen. Specimens in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

Scudder's description is correct. I am not able to decide whether the specimens represent a male and a female of the same species.

Epeira longimana n. sp. (*Textfigure 34*).

One rather poorly preserved male from Florissant, No. 16378 (now No. 123) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

The specimen is complete except for a small fraction of one chelicera and of the side of the abdomen. Total length—7.3 mm., Abdomen—4 mm. long, 3 mm. wide in its anterior third. It is typical of the shape of recent species of Epeira. The palpi are not clearly discernible, nor can the separation line between the carapace and cheliceræ be demonstrated. Although the spider represents the ventral aspect, the sternum is not discernible, nor is there any trace of the edge of the carapace. The shape of the abdomen is as represented in our figure, but at first glance one sees only the darker area. It is the presence of brown hair on the abdomen, which helps to establish the true outline of the latter. There is similar hair on the legs, together with some distinct spines. The legs are in order 1243. The characteristic feature of this species is the proportion in the length of the tibiæ of the first and second leg. As shown by the measurements these tibiæ are considerably shorter than the corresponding femora, a condition unusual in *Epeira* and possibly of a generic value.

Measurements of legs: First leg, femur—5.2 mm., tibia with patella—3.8 mm., metatarsus with tarsus—6.0 mm., total—15.0 mm.; Second leg, femur—3.8 mm., tibia with patella—2.8 mm., metatarsus with tarsus—4.2 mm., total—10.8 mm.; Third leg, femur—1.8 mm., tibia with patella—1.7 mm., metatarsus with tarsus—1.8 mm., total—5.3 mm.; Fourth leg, femur—2.7 mm.,

FIGURE 34.—*Epeira longimana* n. sp. Specimen No. 16378 (123). x 5.

tibia with patella—3.0 mm., metatarsus with tarsus—2.8 mm., total—8.5 mm.

Nothing else can be made out.

Epeira indistincta n. sp. (*Testfigure 35*).

One male from Florissant, No. 14986 (now No. 122) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

There seems to be little doubt as to the sex of this specimen, although the shape and size of the abdomen is rather unusual and more typical of recent females. Yet one can see two dark impressions in front of the sternum, having more the appearance of the palpal bulbs than of cheliceræ. The spider presents its ventral

FIGURE 35.—*Epeira indistincta* n. sp. Specimen No. 14986 (122). x 5.

surface and is very poorly preserved. It is the faintness of the outline of its abdomen, which induced me to give to this species the name *indistincta*. At first one sees only the darker area behind the hind femora and the spinnerets. On careful examination however one notices the true shape especially since the abdomen is clothed with very short brown hair which is more or less plainly discernible under higher power.

Total length 5.7 mm. The legs are very incompletely preserved and cannot be measured satisfactorily. It is evident, however,

that their order is 1243. The first femur is heavy, the fourth longer than the first, but considerably more slender. The third femur is quite short. The legs of the third pair are bent across the abdomen. All legs are thickly covered with short hair, but there are only a few spines discernible. The spinnerets appear as a dark disc considerably in front of the posterior end of the abdomen and seem to be circumvallated as in the preceding species.

Epeira sp.

Scudder, Tertiary Insects p. 88. Plate 11, fig. 1.

An incompletely preserved specimen No. 9285 (now No. 113) from Florissant in the Collection of the Museum of Comparative Zoology of Harvard University. All that can be made out has been described by Scudder. The generic affiliation is very uncertain.

Epeira sp.

Scudder, Tertiary Insects, 1890, p. 89.

Scudder mentions this specimen, No. 63 from Green River, Wyoming. I have not seen it, but the few words which Scudder uses for its characterization are sufficient to show that the specimen is too poorly preserved to be of any value.

FAMILY LYCOSIDÆ.

Characters of the Family:—Eight eyes in three rows, all diurnal. First row composed of four small eyes. Eyes of the second row largest, directed forward, those of the third row directed upward. Posterior piece of the lorum of the petiolus emarginate in front. Trochanters notched. Claws 3.

Genus *Lycosa* Latreille.

At present the genus *Lycosa* is used by one group of Arachnologists with Simon as their spokesman for species referred by the other group to the genus *Tarentula* which two genera are therefore synonymous. On the other hand the second group uses the name *Lycosa* for spiders which Simon places under the genus *Pardosa* C. Koch. I am accustomed to follow Simon's definition.

The only spider which I refer to the genus *Lycosa* has the external appearance of recent representatives of the genus. This is, however, as far as one can go in placing the spider and in this paper I use therefore the genus *Lycosa* in an indeterminate sense.

Lycosa florissanti n. sp. (*Textfigure 36*).

One female, Nos. 10234 and 10240 in the Collection of the American Museum of Natural History.

FIGURE 36.—*Lycosa florissanti* n. sp. Specimen No. 10234. x 3.33.

Except for the ends of the first and second pair of legs the specimen is complete and is well preserved. Total length including cheliceræ—8.0 mm. The carapace is three mm. long, oval in shape, slightly narrowed in front. The eyes are not discernible. The abdomen is elliptic, evenly rounded in front and in the rear, 4 mm., long, 3 mm. wide. The cheliceræ are powerful. On the reverse one can clearly see their entire outline, the sternum and the coxæ, the posterior pair of which is contiguous. The spinnerets are visible but the details cannot be made out. The epigynum can also be seen and reminds one of the similar organ in recent

species, although no detail is discernible. The third and fourth right trochanters show a notch at their distal ends. Body and legs are thickly clothed with simple brown hair. A few spines can be seen on the legs and palpi, but their arrangement cannot be determined. Measurements of legs:—First leg, femur—2.8 mm., tibia with patella—3.6 mm.; Second leg, femur 3.0 mm., tibia with patella—3.3 mm.; Third leg, femur—2.8 mm., tibia with patella—2.8 mm., metatarsus with tarsus—3.2 mm., total—8.8 mm.; Fourth leg, femur—3.0 mm., tibia with patella—3.7 mm., metatarsus with tarsus—4.0 mm., total—10.7 mm. From these measurements it would appear that the fourth leg is in all probability the longest. The femora are heavy, those of the first and second pair appreciably distended in the middle. The pedipalpi are slender, 3.0 mm. long. No claws can be seen, nor can anything more be exposed.

ORDER OPILIONES.

Characters of the order:—Abdomen segmented, broadly joined to the cephalothorax. Cheliceræ three-jointed, chelate. Pedipalpi not chelate. Coxæ of pedipalpi and of the first and second pair of legs with maxillary lobes (gnathobases). Tarsi of variable number of joints. Eyes two. Respiration by means of tracheal tubes with one pair (sometimes with two pairs) of stigmata.

The separation of the order into sub-orders is based on the relative position of the coxæ.

Genus *Phalangium* Linnaeus.

This genus belongs to the sub-order Plagiostethi of Simon, or Palpatores of Thorell. Nevertheless I am using it here in an indeterminate sense, merely as representative of the order or at best of the sub-order.

Phalangium oculatum n. sp. (*Textfigure 37*).

One specimen, No. 9494 (now No. 121) in the Scudder Collection of the Museum of Comparative Zoology of Harvard University.

Total length—3.8 mm. Abdomen 2.4 mm. wide, with a darker band in the middle and two short, pointed spines near the poste-

rior end. Two circular eyes, separated from each other by their diameter, are situated on a transversely oval field. This field is quite flat and it is not probable that in life it had the shape of a tubercle. The chelicerae are scarcely visible. The pedipalpi are

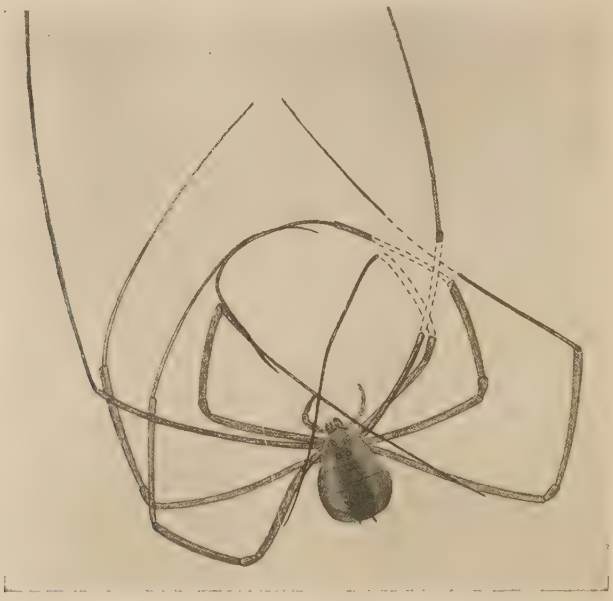

FIGURE 37.—*Phalangium oculatum* n. sp. Specimen No. 9494 (121). x 3.33.

short. The legs are very long. Those of the second pair are broken off and even in the left leg, which is more complete than the corresponding right leg, it is not possible to determine how much of the leg is missing. The right first leg is complete except for the tibia, the right third leg complete except for a piece of the metatarsus and the right fourth leg complete except for a short piece on each side of the tarso-metatarsal joint. The legs of the left side are complete except for the end of the second leg. Measurements are difficult owing to the curvature of the joints and are therefore only approximate. First leg, femur—4.7 mm., tibia with patella—4.6 mm., metatarsus with tarsus—12.6 mm., total—21.9 mm.; Second leg, femur 8.0 mm., tibia with metatarsus and tarsus (incomplete)—17.0 mm., total (incomplete) 25.0 mm.;

FIGURE 38.—*Phalangium lacoei* n. sp. Specimen No. 38124. x 2.5.

Third leg, femur—5.8 mm., tibia with patella—5.7 mm., metatarsus with tarsus—8.4 mm., total—19.9.; Fourth leg, femur—6.8 mm., tibia with patella—6.0 mm., metatarsus with tarsus—16.5 mm., total—29.3 mm.

It is not possible to determine the sex of the specimen.

Phalangium lacoei n. sp. (*Textfigure 38*).

One specimen No. 38124 in the R. D. Lacoe Collection of the U. S. National Museum.

This is a considerably larger species than the preceding one and reminds one greatly of the common recent species of *Liobunum*. The dorsal surface alone is visible, but no traces of segmentation can be made out. Neither are the eyes discernible. A broad median longitudinal band extends from the anterior to the posterior end of the body. The body is clothed with scattered short stout hair. The fourth legs and the left third leg are complete. The others are broken off as represented in our figure. The right second leg is misplaced in such a way that it comes to lie between the third and fourth legs, the former being directed forward. It may be recognized however by its heavier femora. Total size—6 mm., width in middle 4 mm.

First leg, femur—4.0 mm., tibia with patella—5.5 m.; Second leg, femur—6.8.; Third leg, femur—8.3 mm., remaining joints—16.2 mm., total—24.5 mm.; Fourth leg, femur—8.0 mm., remaining joints—24.0 mm., total 32.0 mm.

The sex cannot be determined and nothing else can be made out.

APPENDIX.

LEGS OF UNCERTAIN ARACHNIDS.

Scudder has described five legs representing five separate specimens, Nos. 3, 4a, 36, 4199 and 4200. He considered these fragments as belonging to some species of the genus *Epeira*. I have not seen his specimens 4a and 36. Of the other specimens No. 4199 (now No. 115) in the Collection of the Museum of Comparative Zoology of Harvard University is excellently preserved and is reproduced in our *textfigure 39*. The coxa and trochanter are missing. The femur is 6.4 mm. long with two rows of spines of about 12 spines in each row. The average length of these spines

is about 0.5 mm. The patella is exceedingly short. The tibia together with the patella is 7.5 mm. long. There are two rows of about fourteen spines each on the tibia. These spines are longer than those on the femur, the third spine from the base

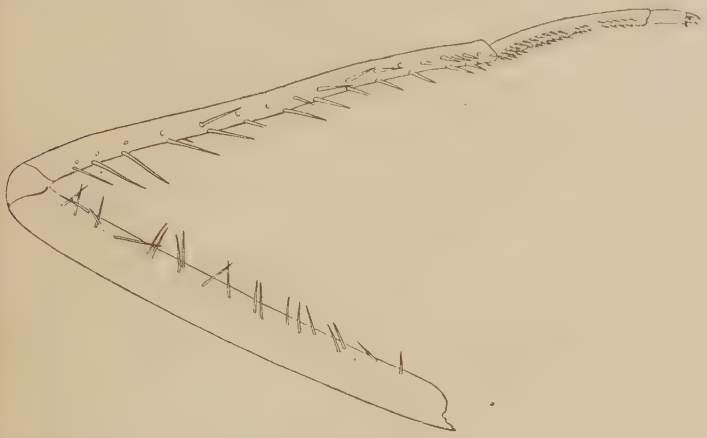

FIGURE 39.—*Leg of an Arachnid.* Specimen No. 4199 (115). x 10.

measuring 1.0 mm. Several spines of one of the rows are missing, but their bases of attachment are clearly discernible. The metatarsus is slightly curved 2.3 mm. long with two rows of about 20 spines each, the spines being very short. The tarsus is only 0.7 mm. long, without spines but with two terminal claws. Each claw is smooth, but with a basal, short, conical tooth. There is no trace of hair on the leg.

The leg is plainly not that of an *Epeira,* nor do I know of any spider with an arrangement of spines similar to this, for these spines are distinctly limited to the ventral surface. The other two specimens, now Nos. 114 and 116, are not complete, but apparently belong to the same species.

COCOONS OF SPIDERS.

Scudder has described and figured cocoons of spiders, referred by him to the species *Aranea columbiae.* (Tertiary Insects, 1890, p. 71, Plate 2, figs. 1 and 2.) I have not seen his specimens, but judging from his description and figures one would suspect that the cocoons in question belong to more than one species. It would of course be impossible to determine the species.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 27

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THE CIRCULATORY SYSTEM AND SEGMENTATION IN ARACHNIDA

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TWO TEXT FIGURES AND TWO PLATES (SEVEN FIGURES)

The circulatory system in Arachnida has been made repeatedly the subject of studies and is fairly well known. Nevertheless, several points have escaped observation, partly on account of technical difficulties, partly because the attention of the investigators was directed toward other aspects of their study. Thus it came to pass that the relation of the circulatory system to the problem of segmentation in arthropods received less attention than it deserves. Indeed, in this respect the circulatory system may be more valuable than the nervous system and may, with a certain portion of the alimentary canal, of which I shall speak in a later contribution, help to establish definite homologies within the phylum of arthropods, and thus not only clear the relationships between the various classes belonging to that phylum, but also throw light on their phylogeny.

Many years ago, in the spring of 1905, while on a trip to Jamaica, West Indies, I collected scorpions and spiders, preserving them in the only fluid then obtainable in Jamaica, a mixture of alcohol with ether. The specimens were simply thrown into a jar and left in the fluid, as I did not intend to use them for any anatomical or microscopical study. Quite recently I wanted a few sections through scorpion embryos for class demonstration, so I imbedded and sectioned some quite young scorpions which had been carried by their mother on her back and of which I had many specimens representing stages before and after the first moult. I also took embryos out of the uterus. My surprise was great when I found that the fixation of the tissues was

remarkably good and that prolonged sojourn in the preserving fluid made the material considerably less brittle than it usually is on account of the voluminous yolk. A cursory examination of the sections has revealed so many interesting deviations from the usually accepted descriptions of the anatomy and embryology of scorpions, that a number of carefully oriented and sectioned series of various stages were made. All sections were purposely made 20μ thick. Exact orientation for sagittal, frontal, and transverse series was comparatively simple on account of the size, shape, and coloration of the material. A few of the series are absolutely symmetrical. The most satisfactory staining proved to be haematoxylin followed by orange G.

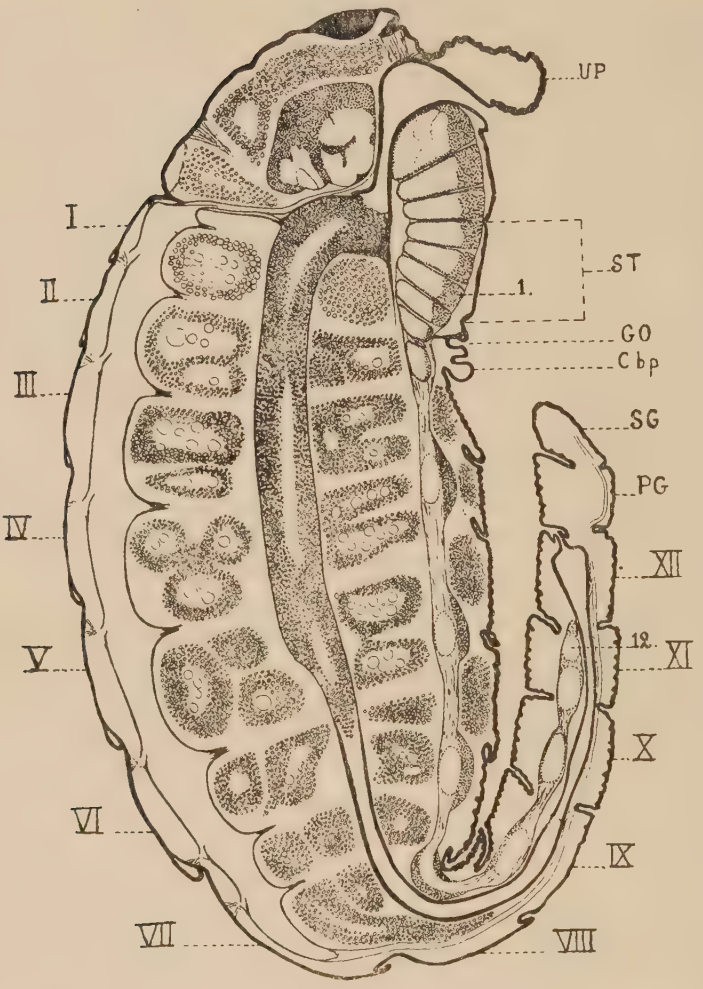
When the study of the circulatory system revealed the remarkable similarity in this respect between scorpions and spiders, I prepared corresponding series through very young spiders fixed for the purpose in my sublimate mixture. Two species of scorpion (Centrurus insulanus and C. carolinianus) and three species of spiders (Agelena naevia, Lycosa carolinensis, and Pholcus phalangioides), belonging to three different families, form the basis of the present study. While it would be very interesting to extend it over other groups of Arachnida, the diversity of the material leaves no doubt that similarity here is not accidental, but is the expression of true homology, and that generalization is therefore warranted and helpful.

To avoid possible misinterpretation of results, obtained only from the study of sections however perfect, a few scorpions were injected through the heart. These scorpions, Centrurus carolinianus of Texas, were obtained alive through the courtesy of Professor Painter, of the University of Texas, who kindly took the trouble to collect and mail to me a dozen specimens. Injection was made in a manner similar to that which I used for Lycosa several years ago, but the technique in the case of the scorpion is somewhat more complicated, owing to the fact that the heart is only imperfectly visible through the chitin. For this reason it becomes necessary to open the chloroformed specimen in a saline solution to expose the heart. The tergites of the second to sixth abdominal segments are carefully removed,

beginning with the posterior edge of the sixth tergite. The ligaments of the heart are cut close to the hypodermis with a sharp scalpel, as otherwise the heart would sustain injury. Freshly precipitated carmine as injection fluid proved to be quite satisfactory. Not only the large vessels become injected to their end near the base of the claws in the legs, but many ramifications of pedal arteries appear dark red. The injected specimen is next fixed in 95 per cent alcohol, dehydrated in absolute alcohol, and cleared in cedar oil, in which it becomes sufficiently transparent for further preparation. All organs obstructing the view are now carefully removed with the aid of two needles under a binocular dissecting microscope and the entire circulatory system exposed to view.

Usually the circulatory system in scorpions is described as consisting of a dorsally situated heart which gives rise anteriorly to the cephalic aorta and posteriorly to the posterior aorta. The heart itself is said to consist of eight chambers with a pair of ostia each, or eight pairs of ostia altogether, typically one pair for each segment of the body. The cephalic aorta is described as giving rise to a pair of arteries near its base, and a little further to another pair. The latter assume a downward course, pass on each side of the oesophagus, forming a ring from which six pairs of arteries are said to be given off to the appendages, while a single median supraneural artery runs from the ring backward above the nervous system. The usual description of the finer ramifications, as well as of the arteries given off by the heart, is irrelevant to our purposes and may be entirely omitted.

The microscopical structure of the heart seems in all Arthropoda to be more or less the same. Its wall is composed of three layers. The outer layer, the adventitia, consists of connective tissue. The heavy media or muscularis is formed either by a spiral muscle or by symmetrically arranged semicircular muscle fibers which meet in the middorsal and midventral line, as has been described by Bergh for insects. The inner layer or intima is, whenever present, nothing but a very thin transparent membrane which, according to Verson, may be nothing but the sarcolemma of the muscle fibers of the media.



Text figure A. Centrurus insulanus, late embryo. Sagittal section in the plane of symmetry, showing all organs developed at that age. The black spot above is one of the median eyes. In front of the eye is the dorsal dilator of the pharynx. Abdominal tergites marked with Roman numerals *I* to *XII* without reference to their embryological history. *1*, the first abdominal neuromere; 12, the last neuromere; *Cbp*, basal plate of comb; *GO*, genital opening; *PG*, poison gland; *SG*, sting; *ST*, sternum; the dotted lines showing its anterior and posterior margins; *UP*, upper lip (rostrum). Further explanation in the text.

This description of the gross anatomy of the circulatory system is, as we shall see, not quite correct, but served to show the similarity between the scorpion and Limulus. On the other hand, it created differences between scorpions and spiders especially, since the circulatory system of the latter had only recently been described correctly. Moreover, the exact position of the heart in both groups remained unnoticed, and yet therein lies its value from the morphological point of view.

The best material for the study of the circulatory system in the scorpion is furnished by that stage of embryos represented in our text figure A, in which all organs are already fully developed, but the nervous system has not yet reached its final state of contraction. The postabdomen is still short, the length of each segment being smaller than the diameter. The last neuromere has already begun to fuse with the preceding one and is almost entirely withdrawn into the fourth postabdominal segment. The first and second abdominal neuromeres have moved forward, passed the diaphragm, are completely within the cephalothorax, and are fused with the thoracic ganglia into one mass in which the separate neuromeres remain however clearly defined as they do, even in adult scorpions. The third abdominal neuromere is just on the verge of passing the diaphragm, while the fourth, which in the adult scorpion forms the last portion of the thoracic ganglionic mass, is still in the abdomen just behind the genital opening and on a level with the basal plate of the comb. In this connection we may state that Buxton had recently shown that the comb receives its nerves from the eighth postoral ganglion of the suboesophageal mass, i.e., from the third abdominal neuromere—an observation which I am able fully to confirm.

In median sagittal sections the diaphragm appears as a thin line. Its ventral portion runs from the ventral body wall just in front of the genital opening to the endosternite which lies above the nervous system. Its dorsal portion arises from a vertical transverse crest of the endosternite and proceeds upward to the dorsal body wall, where it is attached between the carapace and the first abdominal tergite.

The midgut may easily be recognized because of the presence of a cardiac valve, because of its thin walls and, further, because it is still filled with embryonic yolk. It has the appearance of a wide tube with larger lateral branches or diverticula usually known under the name of 'liver.' The anterior portion of the midgut proper runs at almost a right angle to the longitudinal axis of the body and belongs to the cephalothorax because it lies in front of the diaphragm. Its posterior end extends only to the end of the fifth abdominal segment. The diverticula of the midgut extend forward almost to the anterior end of the cephalothorax and backward through the entire preabdomen, the last dorsal diverticulum reaching even into the first post-abdominal segment. The gross anatomy and segmental arrangement of the diverticula are not easy to understand. Sagittal sections show a clear separation of the diverticula into ten metamerically arranged groups, two of which are in the cephalothorax and eight in the preabdomen. Their metameric nature is especially emphasized by the dorsoventral muscles and the hypocardiac ligaments. The ligaments shown in text figure A as ventral projections of the heart exist only in the preabdomen. Of the muscles, one pair is in the cephalothorax where they are attached by one end to the carapace between the two cephalothoracic diverticula and by the other to the endosternite above the nervous system. There are eight pairs of dorsoventral muscles in the preabdomen passing between the diverticula, right and left of the midgut, from the back to the ventral surface of the body. The first pair is easily overlooked as it lies closely applied to the posterior surface of the diaphragm. Dorsally, these muscles are attached to the anterior end of the first abdominal tergite on the outside of the epicardiac ligaments. Ventrally, they are attached, like the cephalothoracic pair, to the endosternite above the nervous system. All other dorsoventral muscles are attached at both ends to the chitin of the body wall. The dorsal attachments are to the outside of the epicardiac ligaments. The ventral attachments are slightly farther apart than the dorsal, one pair for each abdominal sternite, the second pair lying at the sides of the genital opening, and the third at the sides of the basal plate of the comb.

Although the diverticula are divided by the dorsoventral muscles into ten groups, there are only six pairs of ducts connecting them with the midgut proper. The first pair of these transverse ducts is in the cephalothorax and connects the two cephalothoracic diverticula with the anterior portion of the midgut. The first, second, third, and fourth abdominal diverticula have a pair of transverse ducts each in the corresponding segments. The last pair of connecting ducts opens into the midgut in the fifth abdominal segment. These ducts are long, extending backward over three segments and establishing a connection between the midgut and all four posterior metameric groups of diverticula.

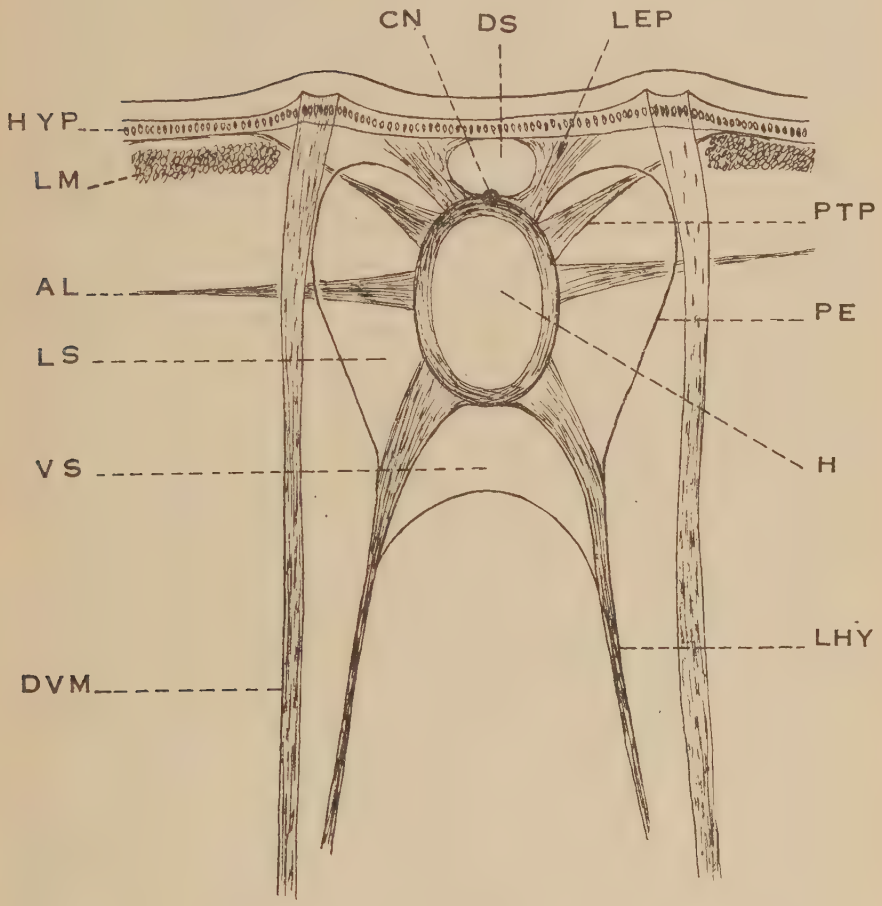
Transverse sections through the second to fourth abdominal segments show that each pair of diverticula is composed of two dorsal, two ventral, and one lateral lobes. All lobes are filled with embryonic yolk and all those on the same side are naturally connected with each other near their base. The dorsal right and left lobes are separated by the pair of dorsoventral muscles. Below the midgut proper, which occupies approximately the center of a transverse section, the dorsoventral muscles pass on the outside of the inner pair of ventral lobes. In the cephalothorax only dorsal lobes are present. The last pair of transverse ducts gives off side branches to the fifth and then to the sixth abdominal diverticula, while the ends of the ducts lead into the seventh pair into which the eighth pair also opens.

The hindgut begins in the terminal region of the fifth abdominal segment. It is considerably smaller than the midgut, but has both relatively and absolutely much thicker walls. It is devoid of embryonic yolk and in the last postabdominal segment forms a considerable widening beyond which it is again suddenly constricted and opens with a small anus in the midventral line at the end of the segment.

The heart lies entirely in the preabdomen, extending from the diaphragm which separates the latter from the cephalothorax, almost to the end of the seventh abdominal tergite. Its anterior and posterior limits are clearly defined by the valves of which I shall speak later. The heart has seven pairs of ostia

(not eight as usually erroneously stated). The presence of the ostia gives the heart the appearance of an eight-chambered organ. In reality there are neither valves nor any constriction or impediment in the spaces between the successive ostia. There is, therefore, nothing that would have the morphological value of chambers. In transverse sections the ostia occupy a position approximately half-way between the dorsal pole and the equator of the heart. The first pair lies exactly at the posterior edge of the first abdominal tergite. The position of all seven pairs may be best understood from text figure A. It is the same in all specimens and does not change with maturity. The structure of an ostium with its valve is represented in figure 3 on a large scale. This is a frontal section, and the media or muscularis is therefore sectioned at right angles to its muscle fibers. Each fiber has the appearance of a rectangle. The adventitia of connective tissue is well defined and shows long, darkly stained nuclei. Multinuclear fibers of connective tissue run from the edges of the ostium laterally, converging and forming a ligament, the so-called pteripyle.

The position of the ligaments by which the heart is suspended is well known in spiders, owing to several researches, especially those of Causard. There is scarcely any difference in this respect between spiders and scorpions. Text figure B, drawn from a complete series of transverse sections through a late embryo, represents the heart of the scorpion with all its ligaments of a single group projected into the same plane. All told, there are eight metamerically arranged groups of ligaments, each group, except the first, composed of four pairs. The shortest of these are the epicardiac ligaments which pass on each side of the dorsal sinus and are attached to the basal membrane of the hypodermis, thus clearly demonstrating their connective nature, since all muscular fibres, as for example those of the dorsoventral muscles represented in the figure, pass between the hypodermal cells and are inserted in the base of the cuticle. The second pair are the pteripyles. Their distal end merges with the somatic connective-tissue layer which separates the dorsal longitudinal muscles of the abdomen from the hypodermis. The third pair,



Text figure B. Centrurus insulanus, late embryo. Transverse section through the heart in the region of the third abdominal segment, showing the ligaments. The drawing was made from two sections, as not all ligaments are in the same plane. The epi- and hypocardiac ligaments are in one plane, while the pteripyles and alar are ligaments in another. *AL*, alar ligaments; *CN*, cardiac nerve; *DS*, dorsal pericardial sinus; *DVM*, dorsoventral muscle; *H*, heart; *HYP*, hypodermis; *LEP*, epicardiac ligaments; *LHY*, hypocardiac ligaments; *LM*, dorsal longitudinal muscles; *LS*, lateral pericardial sinus; *PE*, pericardium; *PTP*, pteripyle; *VS*, ventral pericardial sinus.

often called alary muscles, are the alary ligaments. Their ligamentary nature has been elucidated by Causard. They are directed at almost right angles to the longitudinal axis of the heart. Distally they are not attached to the body wall, as usually stated, but merge with a layer of connective tissue, evidently representing the splanchnic coelom covering and separating the diverticula of the midgut from other organs situated above the latter. The fourth pair are the hypocardiac ligaments. They are by far the strongest and longest, and are easily mistaken for muscles, especially where they intercross with the dorsoventral muscles. From here on they continue diverging and unmistakably and finally merge with the splanchnic layer of connective tissue which covers the diverticula of the midgut from below. The first group of ligaments consists of two pairs only. The epicardiac ligaments are attached to the anterior edge of the first tergite. The hypocardiac ligaments are more or less normally developed, but the pteripyles and alary ligaments are wanting.

Since there are no muscles for the dilatation of the heart, diastole is accomplished through the elasticity of the heart ligaments. This explains why the muscularis of the heart is so powerfully developed. During systole the heart has to overcome the resistance of the ligaments, while the contraction of the latter during diastole is not impeded by the relaxed muscles. There is nothing unusual in such arrangement, as a similar condition exists in almost all joints of the appendages in Arachnida, where flexing is accomplished by muscular contraction and extension by the elasticity of the interarticular chitinous membrane. I have counted 120 pulsations of the heart in one minute.

The pericardium appears as a thin membrane, and the space between it and the heart is, in sections, invariably filled with coagulated blood plasm, and consequently is clearly discernible. Owing to the presence of epicardial and hypocardial ligaments, this space is subdivided into four regions which may be termed sinuses, though they communicate with each other in those regions of the heart where there are no ligaments. The lateral sinuses are the largest, next in size is the ventral sinus, while the dorsal sinus, almost round in shape, is the smallest of the four.

In the dorsal midline of the heart, partly imbedded in a groove in the wall of the heart, the cardiac nerve extends from one end of the heart to the other (text figure B, *CN*). The nerve is clearly visible in all transverse sections and unquestionably corresponds to the cardiac nerve described in Chilopoda (Duboscq), Protracheata, and other Arthropoda. As my material is not specially prepared for the study of nerves, I am unable to find a connection of the nerve with the brain, but such connection has been described by Police in Euscorpius.

The structure of the anterior aortic valve is best understood from median sagittal sections and sections which traverse the valve more or less at right angles. In the first (fig. 1, *AV*) the valve appears as a line attached to the dorsal wall of the heart exactly under the epicardiac ligaments, inclined downward, and about two and a half times as long as the diameter of the heart at the place of the attachment of the valve. In reality the valve is a muscular membrane arising from the dorsal half of the wall of of the heart and attached to the sides of the vessel throughout its length. The anterior edge of the valve is longer than the diameter of the vessel. The valve has, therefore, a peculiar shape, being concave or troughlike at its free edge and convex or arched at its base. About half-way between its base and end the valve is drawn tight in the equator of the transverse section of the vessel. Such a section is represented in figure 4, which also shows that the valve is not a fold, but consists of a single layer of transverse muscular fibers with elongated nuclei. There is always a greater accumulation of blood-cells above the valve than below it, showing that the action of the valve is perfect.

The structure of the posterior aortic valve is more difficult to ascertain, and is somewhat different from the anterior one. Text figure A represents the position of the posterior valve as being not far from the posterior edge of the seventh abdominal tergite. This position is constant in specimens of all ages. The valve seems to have the shape of a cone, the open free apex of which is directed posteriorly, while the broad base is attached to the wall of the heart along its entire circumference. This valve, too, has a single layer of circular muscle fibers composing it,

but the length of the posterior valve is many times smaller than that of the anterior valve. Indeed, the posterior aorta which begins at this place is a thin vessel gradually becoming smaller as it traverses all the segments of the postabdomen. It may be traced through the poison gland into the sting, where it ends apparently without any ramifications.

I have stated that the valves are muscular in structure. It may be objected that I have adduced no evidence in support of this assertion and that one may just as well claim that the valves are internal projections of the same connective tissue which as its adventitia surrounds the heart. Indeed, I have no sections through either the anterior or posterior aortic valves to prove or disprove either of the contentions. But I have already mentioned the fact that spiders possess the same types of valves. A comparison of figure 6 with figure 1 will show that the position and appearance of the anterior valve in both scorpions and spiders is the same. Similarly, a comparison of figures 4 and 7 will disclose the identity in structure of the anterior aortic valve in these two orders of Arachnida. Now I happen to have a great many sections through young spiders, and these show the intima lining both surfaces of the valve and continuing directly as intima of the heart itself. In many cases there is a slight loosening of the intima from the muscularis, with the consequence that it appears as an uninterrupted line. The intima of the heart being the sarcolemma of the muscle fibers, it is not conceivable that it could line any but muscular tissue.

Let us now turn our attention to the anterior aorta and the arterial blood vessels of the cephalothorax (fig. 1). The anterior aortic valve, having the exact direction of and lying immediately in front of the diaphragm represents the exact demarkation line between the heart and the aorta. Otherwise, the transition from the heart to the aorta would be scarcely perceptible. Shortly beyond the valve the aorta gives rise to a pair of small arteries supplying with blood the pair of dorsoventral muscles which separate the first cephalothoracic diverticula of the midgut from the second.

The aorta itself continues as a considerable vessel under the brain until it reaches the third neuromere of the suboesophageal ganglionic mass. Here the aortic arch around the oesophagus is formed. The arch is very short and connects the aorta with the right and left thoracic sinus opposite the base of the second pedal artery as shown in figures 1 and 2. Two pairs of vessels issue from the aortic arch. The first pair are the large cephalic arteries shown in figure 1. The second pair cannot be shown to advantage in the planes chosen for our drawings and is therefore not represented there. But these vessels are nevertheless constant in their position and easily found. Their roots are in the right and left anterior inner edge of the aortic arch, near its junction with the sinus and almost directly above the third vessel connecting the sinus with the supraneural artery. The two vessels run convergingly upward, feeding the wall of the oesophagus.

Each thoracic sinus gives rise to small and thin vessels connecting the sinus with the supraneural artery, and to four large vessels for the appendages. Of these vessels the first is the largest and splits almost immediately into two branches. The outer branch (fig. 1 and 2, *1*, *AP*) is the first pedal artery, while the inner, longer, and stouter branch is the pedipalpal artery (*APP*). The latter gives off a thin branch directed inwardly, following in its course the curvature of the ganglionic mass, and connecting with the supraneural artery just behind the pharynx. A branch of this supplies the tissues in front of the pharynx.

Posteriorly, the right and left sinus merge with each other and form a connection with the supraneural artery. This artery is single and runs in the median line above the ventral nervous system and closely applied to it. Anteriorly it runs to the very end of the ganglionic mass, turning downward in its course and now continuing backward in the midventral line below the ganglionic mass as subneural artery. Posterior to the thoracic sinus the supraneural artery continues as a straight vessel in the median line above the nervous system and closely applied to it through the entire abdomen. I have not followed its course in the postabdomen.

Nine single interneural vertical arteries connect the supraneural with the subneural artery. These vertical arteries pass exactly between adjoining neuromeres, the first artery separating the pedipalpal from the first pedal neuromere, the ninth lying immediately behind the fourth abdominal neuromere of the suboesophageal ganglionic mass. Median longitudinal connecting vessels seem to exist between all vertical arteries, but only between the fifth and sixth, and between the sixth and seventh vertical arteries the connecting vessels are invariably well discernible, as shown in figure 1.

The subneural artery does not extend beyond the suboesophageal ganglionic mass, but ends behind the fourth abdominal neuromere, where it receives the ninth vertical artery, which may be, therefore, in a way regarded as a direct continuation of the subneural artery. At the place of their junction a single blood vessel is given off ventrally. This is the comb-artery shown in figures 1 and 5. It gives off a pair of branches, one for each comb, and another pair of smaller branches for the genital opercula.

Each cephalic artery gives off several branches, the most important of which is the ophthalmic artery shown in figure 1. Beyond the ophthalmic artery the main vessel may be termed cheliceral artery. Inside the chelicera the cheliceral artery forms two ramified branches, one for the flexor and the other for the extensor of the movable finger.

COMPARISON WITH SPIDERS

Having thus described the most important features of the circulatory system in scorpions, we now may proceed to its comparison with the corresponding system in spiders. A glance at plate 3 of my paper published in the Zoologische Jahrbücher for 1920, vol. 31, will reveal both the similarities and diversities of structure. What I described there as 'Kopfarterie' corresponds with the cephalic artery of the scorpion almost to the minutest details, and shows the same ramifications. On the other hand, in the spider the cephalic arteries represent the upper branches of the thoracic arteries, the lower branches of which

lead to the thoracic sinuses, whereas in the scorpion the cephalic arteries arise from the aortic arch. This means simply a further extension of the arch in the spider, so that the aortic arch of the scorpion corresponds with the thoracic and connecting arteries of the spider. The homology is unmistakable, and it may be wiser to speak in the spider also of an aortic arch instead of a thoracic and a connecting artery.

The thoracic arch, then, of the spider opens into the thoracic sinus at the base of the second pedal artery as in the scorpion. As in the scorpion, the pedipalpal and the first pedal arteries are branches of the first arterial stem given off by the thoracic sinus. The aorta recurrens of the spider, shown in my drawings, is the supraneural artery. But for the comparison of the supraneural and subneural arteries of the scorpion and spider we have to consult the description of these arteries given by Causard, and his figures on plate IV. Instead of quoting passages in the original, I translate them with such omissions as have no relation to our subject.

"We will now consider the arteries which issue from the aortic arches. For a long time two roots were described issuing from the posterior end of each goose-foot [my thoracic sinus,—*A. P.*], forming by their junction a sort of supraganglionic anastomosis which gives rise to a longitudinal artery directed backward and running along the dorsal surface of the ganglionic mass. Schneider gave this artery the name *supraneural.* He has also shown that in front of this anastomosis there are five others. There are therefore altogether six anastomoses which this author describes as thin and delicate. This is true of the five anterior ones, but cannot be accepted as characteristic of the last one, which has a considerable diameter. Moreover, the supraneural artery is rather stout; how could it be fed by two such fine roots? These, as he shows, are often incomplete, the supraneural artery arising from a single root which may be either the right or the left one. When the root is complete this anastomosis has the shape of a V.

"The anterior anastomosis has the shape of a V open posteriorly [i.e., of an angle with a vertex directed forward—*A. P.*].

It is situated immediately under the oesophagus and gives rise anteriorly to a thin artery which is closely applied to the inferior surface of the oesophagus. This is the *suboesophageal* artery. The four following anastomoses are rectilinear and each gives rise to a vessel which issues from the middle of their ventral surface and traverses the ganglionic mass from end to end to its ventral surface. Schneider gave to these arteries the name of *median cerebellar arteries,* as he did in the case of the scorpion. I prefer to call them *ganglionic median arteries* [my vertical or interneural arteries—*A. P.*]. The supraneural artery gives also rise to a certain number of more or less short arteries of this kind, the first being omitted at the beginning of the supraneural artery and corresponding therefore with the sixth supraganglionic anastomosis. I was able to find seven or eight such arteries, thus bringing their total number to 12 or 13 . . . . What Schneider does not mention is the fact that all these arteries connect on the ventral surface of the ganglionic mass with a median longitudinal lacune" . . . . (pp. 61–62).

Although I have no conclusive evidence at this moment, either to confirm or to disprove some of the statements contained in the above quotation, it seems to me that Causard has been misled by imperfect injections. We easily recognize in the suboesophageal artery of Causard that portion of the supraneural artery, which is shown in the scorpion in our figure 2 as *SOA*. But I think that both Causard and Schneider have overlooked the connection of the 'anastomoses' with the supraneural artery. Causard, indeed, has seen their connection with the subneural artery by means of the vertical arteries (ganglionic median arteries). On the other hand, the number of these vertical arteries given by Causard as 12 or 13 seems to be decidedly too great. A careful examination of sagittal series of sections through young spiders shows invariably the presence of eleven distinct neuromeres in the thoracic ganglionic mass. The first belongs to the pedipalpi, the second to fifth to the legs; the tenth corresponds to the same neuromere in the scorpion, which in the latter is already in the abdomen. The eleventh neuromere is imperfectly divided into two. The anterior portion

is the eleventh neuromere proper, while the posterior portion represents the remnant of the abdominal neuromeres, whether contracted and fused or lost altogether makes no difference as regarding our proposition. We thus have at the most eleven interganglionic surfaces, if we count the partition of the eleventh neuromere as complete. Therefore, there cannot be more than eleven vertical arteries, since arteries passing through instead of between ganglia are not known.

The heart of the spider has four pairs of ostia in the Theraphosidae and only three pairs in the true spiders, as against seven pairs in the scorpions. From the position of the aortic valve, it is safe, however, to accept that the reduction in the number of ostia took place in a progressive direction from the rear end of the heart forward. What has happened to the rear portion of the heart, which has lost the ostia? I think it must have shrunk in size, become considerably thinner and changed into what became the proximal end of the posterior aorta. We have seen that the posterior aortic valve has a structure distinctly different from that of the anterior valve. It would be scarcely necessary to assume a progression of the posterior aortic valve, a shifting of its position with the loss of ostia. Is it not more likely that the posterior aortic valve is a modified remnant of the last pair of ostia valves which have become functionless as such, when the ostia themselves closed? With other words, that the posterior aortic valve of a Theraphosid is the remnant of the fifth pair of ostia valves, while in true spiders it is the remnant of the fourth pair?

From the above comparison of the circulatory system of the scorpion with that of the spider we may now draw the following important conclusions: the scorpion represents the more generalized and therefore more primitive circulatory system among Arachnida, the spider the more modified and therefore the more advanced. The most permanent structure in the circulatory system of Arachnida is the anterior aortic valve which is attached at the anterior edge of the first abdominal tergite and therefore marks the limit between cephalothorax and abdomen. The reduction in the number of ostia stands in direct relation-

ship with the loss of segmentation in the abdomen and proceeds in the same direction, that is, from the posterior end forward. The changes in the neural portion of the circulatory system do not extend over the thoracic haemomeres because of the permanency of the thoracic appendages, but follow the changes in the position of abdominal neuromeres. As the contraction of the longitudinal connectives between neuromeres brings abdominal neuromeres into the thorax, abdominal vertical arteries are also shifted in position, while the complete disappearance of the last abdominal neuromeres brought about a corresponding complete disappearance of the last vertical arteries.

COMPARISON WITH LIMULUS

The circulatory system of Limulus has been excellently described by Milne-Edwards, and such errors as he has admitted in his description have been later corrected by Patten and Redenbaugh. I have made injections of adult large specimens to verify the results, and can only confirm their correctness. It is different, however, with the interpretation of the structures, and here I disagree both with the older and later investigators.

Alphonse Milne-Edwards worked eight years before Lankester, and although the idea that Limulus is an Arachnid had been already advanced by Latreille and later by Owen, yet the knowledge was not sufficient to admit of incontrovertible homologies. Consequently, notwithstanding the great similarity in the structure of the nervous and circulatory systems, Milne-Edwards felt justified in pointing out the differences and in refusing to place Limulus either among Crustacea or among arachnids. For reasons which it is not worth while reviewing at present, Milne-Edwards considered the first pair of appendages in Limulus homologous, not with the chelicera, but of the pedipalpi in scorpions.

Lankester's interpretation of Limulus was colored by his theory of tagmata into which (according to him) the body of an arthropod is divided. He finds that the body of Arachnida is composed of three tagmata of six somites each and that the genital openings are placed on the first somite of the second

tagma or mesosoma. Following this idea, he finds the same tagmata in Limulus, the mesosoma being represented by the genital opercula and the five gill-plates, while the metasoma is reduced to a very small area around the anus, including the last pair of lateral spines.

Patten and Redenbaugh do not attempt to change the interpretation of Lankester, but correct it in regard to the chilaria. The presence of a distinct neuromere for this pair of appendages having been established by Kingsley, our authors naturally ascribe to them the value of a distinct metamere and consider the chilarial somite as belonging to the cephalothorax. For them, as for all previous investigators, the articulation between the carapace and the abdomen is the segmentation line separating the cephalothorax from the abdomen.

It would be useless to describe here in detail the entire circulatory system of Limulus, but certain features of it must be considered. The heart occupies the same position as in other arthropods and extends from about the middle of the line passing through the side eyes back to about the middle of the abdomen. The heart has no opening posteriorly and the superior abdominal artery is connected with the heart only indirectly through the collateral arteries. Therefore, though occupying the same position as the posterior aorta of Arachnida, the superior abdominal artery of Limulus cannot be regarded as homologous with the latter. The number of ostia is greater than in the scorpion, inasmuch as Limulus has eight pairs. Patten and Redenbaugh describe and figure a pair of rudimentary ostia in front of the aortic valve. These may be the last remnants reminiscent of a still older time when the ancestor had a heart extending farther forward. The aortic valve has almost the same structure as in Arachnida. In front of the valve "a pair of tendinous bands, comparable to a pair of alary muscles, run forward and upward a short distance beyond the limits of the pericardium, and attach themselves to the carapace close to the insertions of the tergoproplastral muscles" (p. 127). I may add that this connection is so strong that in removing the carapace the heart is easily injured, unless particular care is given to sever the connection of

these tendinous bands, which is certainly not the case with the heart ligaments of the subsequent metameres.

The aorta is exceedingly short and forms almost at once two vessels which are rightly regarded as the aortic arch. These vessels are large and long, run at first forward, then curve downward, pass the oesophagus on each side and open into the 'vascular' ring a little to the inside of and above the base of the first pedal artery. The entire ventral circulatory system of Limulus is perineural; i.e., it sheaths completely the nervous system. Not only the postoral neuromeres of the suboesophageal ganglionic mass, but the supraoesophageal forebrain as well is enclosed in this perineural circulatory system. The haemal sheath extends through the entire length of the ventral nervous cord in the abdomen. Accordingly, neither supraneural, nor subneural, nor interneural or vertical arteries are present. The cheliceral arteries issue from the ventral surface (actual, not morphological) of the vascular ring. In all this Limulus is very different from the scorpion and other Arachnida. Yet the similarity is nevertheless quite striking. If the forebrain portion of the vascular ring were removed, the rest of it would present an identical appearance with the two thoracic sinuses of the Arachnida. The similarity is increased by the existence of five nervous bridges connecting the right and left ganglia of the five pedal neuromeres. These nervous commissures are naturally ensheathed by the corresponding perineural vessels which, therefore, represent the five arteries in the scorpion connecting the thoracic sinuses with the supraneural artery. But in what way could we explain the origin of the scorpion type of neural circulatory system from the Limulus type or vice versa? Has the perineural system broken up into two sinuses and neural blood vessels, or have the latter altogether a separate origin?

The relatively great size of the oesophagus and the position of the forebrain in front of and not above the suboesophageal ganglionic mass in Limulus may have something to do with the differences between this animal and Arachnida. But this position itself is by no means original. Notice the position of the mouth in the middle of the ventral surface of the cephalothorax

and the position of organs in front of the mouth, which morphologically have to be considered as postoral. Notice the plastrobuccal muscles going "from the anterior neural side of the plastron to the oesophagus" and the strands of muscles attaching the proventriculus to the carapace in the region of the median eyes. The former undoubtedly represent the pharyngeal dilators of Archnida, the latter the dilators of the sucking-stomach in spiders and the corresponding pair of dorsoventral muscles in the scorpion. Although considerably in front of the posterior edge of the carapace in Limulus, these muscles are not far in front of the aortic valve. Notice that in severing the carapace from the abdomen with a knife, the opercular plate remains with the carapace. Notice, further, that the suboesophageal ganglionic mass in Limulus consists of seven neuromeres, the sixth belonging to the chilaria and the seventh to the opercula; that, owing to the perihaemal type of blood system, the vessels for the chilaria and opercula issue from the vascular ring; notice all this and you get the idea of what happened to Limulus in the course of its phylogenetic development. On the ventral surface two somites, corresponding to the first and second abdominal somites in Arachnida and characterized by the chilaria and opercula, became fused with the thoracic somites, while at the same time the corresponding neuromeres moved forward and fused with the suboesophageal ganglionic mass. On the dorsal surface a general displacement forward took place. In this displacement two things remained unchanged: the position of the mouth and the attachment points of the foregut and of the heart in the region of the aortic valve. What was above and behind the mouth, with the forward bending of the back came to lie in front and above the mouth. Part of the heart followed the displacement because of the permanent attachment at the aortic valve. Of the tergites, those of the chilarial and opercular somites had to follow the forward motion of the original carapace and were drawn into the hollow of the horseshoe-shaped carapace as it was formed through the forward displacement. These tergites fused with the carapace along their front and sides, but are still visible even in the adult and especially in the so-called trilobite stage of the

young. The cephalothorax of Limulus is therefore the result of fusion of the original cephalothorax with the chilarial and opercular somites, and the articulation between the carapace and abdomen is in reality an articulation between the opercular and first gill somite, or what corresponds to the division line between the second and third abdominal somites in Arachnida, as exemplified by the genital and comb somites in the scorpion. The division line between the last thoracic and first abdominal (chilarial) tergites lies immediately in front of the attachment of the heart, i.e., somewhat in front of the line passing through the two lateral eyes.

The history of this forward displacement and fusion of originally abdominal somites cannot be gleaned from a study of the external segmentation of Limulus embryos. On the ventral surface segmentation is clear, but on the dorsal the first visible segment is already the first gill segment. Something similar may be seen in the scorpion. Here, in the adult, the third abdominal tergite corresponds to the first lung sternite and therefore in reality representing the fourth abdominal somite. The second tergite, corresponding to the comb, represents the third abdominal somite. But the first abdominal tergite is the result of a fusion of the first and second tergites of the corresponding embryonic somites. The external segmentation is clear in young embryos on the ventral surface, and in quite young embryos is at least indicated by the even segmentation of the nervous system as seen in longitudinal sections through these stages. But when segmentation appears in the shape of transverse depressions on the dorsal surface, the first visible abdominal tergite corresponds already with the same tergite of the adult and is therefore already the result of fusion. It may be argued that if in Limulus abdominal tergites fused with the carapace, the same may have happened in the case of the missing first tergite of the scorpion. But this interpretation meets with too many objections. Of these perhaps the clearest is presented in the case of solpugids in which the thorax is still externally segmented.

In my monograph of Palaeozoic Arachnida ('13) I have pointed out that the Xiphosura must have developed independently

of the scorpions. The idea that Limulus is an arachnid as it is usually expressed, or more correctly that the Arachnida have a common ancestor with Xiphosura, must now be completely abandoned. Geologically, Limulus is older than the scorpion and already the oldest limuloid shows the same type of segmentation as the recent. Neither has the idea of Versluys on the origin of gills from lung books any bearing upon the question of origin of Limulus or Arachnida. With a stress on imagination one may derive Limulus from a eurypteroid ancestor, but to derive the latter from originally air-breathing Arachnida on the basis of no other evidence than conjectures which rest on a comparison of gill-plates with lung books and in the absence of any remains of air-breathing Arachnida antedating eurypteroids, seems to be a rather hazardous undertaking.

It may be interesting to mention in this connection that early stages in the embryonic development of scorpions show clearly eighteen postoral neuromeres, the first of which soon passes in front of the mouth and represents the cheliceral somite. The study of preoral neuromeres in the same stages is too complicated to admit of impartial judgment. In later stages, after the passage of the mouth behind the cheliceral neuromere, one may clearly count three pairs of nerves issuing from what appears to be three corresponding neuromeres. The first pair are the optic nerves of the median eyes, the second the nerves of the lateral eyes, and the third the nerves of the upper lip. These nerves are much finer than both optic nerves and can be traced with certainty only in sections parallel to the plane of symmetry (sagittal). The adult scorpion has therefore four preoral and seventeen postoral neuromeres. Five of the latter belong to the thorax and twelve to the abdomen. It happens that the abdomen of the scorpion shows twelve tergites. Yet one should not conclude from this coincidence of figures that each of the neuromeres mentioned belongs to a corresponding tergite. Nothing of the kind. I have already mentioned that the comb receives its nerves from the third abdominal neuromere, as may be easily demonstrated on sagittal and frontal sections. In early stages the neuromeres do not possess longitudinal connectives and

are recognizable without difficulty only because of the constriction between adjoining neuromeres. The last neuromere is clearly situated in the last abdominal segment, and only later moves forward and fuses partially with the penultimate neuromere. There is, therefore, in the adult scorpion an abdominal segment in excess of neuromeres. From an examination of numerous series I have no doubt that it is the first postabdominal or caudal segment and which therefore may have the value not of a true somite, but of an anterior subdivision or segment of the same somite to which the second postcaudal segment also belongs. Here, then, something happened the reverse of the fusion of sclerites in the first two abdominal somites, namely, the subdivision of the sclerite ring of a single somite into two distinct sclerite rings or segments, without a corresponding subdivision of other structures in the same somite.

It may be objected that such formation of pseudo-segments has not as yet been described, either for Arachnida or other Arthropoda, and that it were simpler to accept that the neuromeres really correspond to the visible segments, but in moving forward lost connection with them and began to furnish nerves to the next following. In other words, that the first abdominal neuromere originally furnished the nerves for the genital opercula, lost connection with the latter, and ceded this morphological and physiological function to the second neuromere; that the same happened to the second neuromere in relation to the comb, which now received its nerves from the third neuromere. But this explanation, besides being more complicated, suffers from another weakness. The roots of nerves follow the displacements of their neuromeres, but the nerves themselves obtain their connection with the original appendages, even if some branch of the nerve passes to another somite. This may be seen in Limulus and in many other arthropods. But in the case of the first abdominal neuromere of the scorpion there is not even a considerable or appreciable displacement forward, so that there would be no morphological reason of any kind for a loss of connection with the genital opercula if these belonged to the first neuromere.

We may therefore form the following conclusions regarding segmentation in scorpions and in Arachnida in general. The body of an Arachnid is composed of twenty one somites, to wit: 1 the first ocular (median eyes in the scorpion, anterior median eyes in the spider); 2 second ocular (lateral eyes in the scorpion, eyes with inverted retina in spiders, i.e., anterior lateral, and the four posterior ones); 3 rostral (upper lip); 4 cheliceral; 5 pedipalpal; 6 to 9 thoracic pedal; 10 to 21 abdominal. The first three are originally preoral in position. The fourth or cheliceral becomes preoral during development. The attachment of the heart to the anterior edge of the dorsal wall of the first abdominal somite and the formation at this place of the aortic valve indicate the division line between the thorax and the abdomen. The tenth somite is always rudimentary, having lost its identity in all but its neuromere. The genital opening is on the eleventh somite (second abdominal). A further fusion and ultimate loss of the identity of somites in Arachnida involves the posterior end of their body, beginning with the twenty-first somite and proceeding forward. In some cases, as in the eighteenth somite in the scorpion, secondary or spurious segmentation may take place, which has no relation to the original metamerism. If there be more than three originally preoral somites, these would have to be sought for in front of the first ocular somite.

Turning once more our attention to Limulus, we may first of all consider the homology of the thoracic and abdominal somites established in a way excluding all doubt. The six pairs of appendages belong to the same somites as in Arachnida, the chilaria represent the tenth, the opercula the eleventh somite, the five branchial neuromeres correspond to the twelfth to sixteenth somites, and of the three postbranchial ganglia the last is the result of fusion of the nineteenth to twenty-first neuromeres, if the ancestor of Limulus possessed that many postbranchial somites.

The homology of the preoral somites is more troublesome. Patten and Redenbaugh describe three preoral neuromeres, the olfactory, median ocular, and lateral ocular. Shipley following Carpenter recognizes only two somites, the median ocular and

the rostral. I think we may consider it as fairly conclusive that the median ocular and lateral ocular somites are homologous in Limulus and the scorpion. Whether the rostral somite of the scorpion corresponds with the somite designated as rostral in Limulus by Carpenter, is not so sure, but if it does not, then Limulus must possess just the same some evanescent somite between the lateral ocular and cheliceral. As for the olfactory somite, its homologue in Arachnida would have to be sought in one of those two pairs of obscure parietal ganglia described by Schimkewitsch for tetraneumonous spiders.

In the presence of a perineural circulatory system, in the existence of eight pairs of ostia in the heart, and of a pair of chilarial nerves, Limulus shows evidence of its origin from an arthropod ancestor lower and more primitive than the Arachnida. But in every other respect Limulus shows advanced development different from that in Arachnida and most likely standing in direct relationship to its particular mode of life. It seems as if the older interpretation of Limulus as a descendant of Trilobites must be revived. The shape of the trilobite carapace, the position of the mouth, the probable similarity in the position of the foregut as suggested in figure 24 of Raymond's beautiful monograph, the larval stages showing segments which were interpreted as cephalothoracic, but some of which probably are abdominal tergites drawn into and fused with the thoracic ones, point to a similarity more than casual. At any rate, the problem should be reinvestigated from the new point of veiw.

COMPARISON WITH OTHER ARTHROPODA

We have seen that the aortic valve has a uniform structure and a permanent position in Arachnida, permitting of strict homologies within that class. We have also seen to what conclusions we arrive through the assumption that the rule holds good in the case of Limulus also. One would expect that a structure so permanent in one or perhaps two classes would prove to be the same in the case of all other Arthropoda, if the diverse forms united under this immense phylum are of monophyletic origin. Unfortunately, this is not the case.

In his work on the organs of circulation in Schizopoda, published in 1883, Delage writes (I translate the original): "To determine exactly the length of the heart one should first of all well define its limits. It happens that these limits are not easily traceable because the diameter of the heart is not greatly different from that of the aortae at their points of origin. (Delage has in mind both the anterior and posterior aorta.) They are marked by the presence of cardioaortic valvules which have not yet been described by anyone. Moreover, these valvules are identical with those which are found in the same place in Amphipoda." "Within these limits the heart extends from the level of the last maxillary segment to the superior portion of the last thoracic segment." The anterior aorta, the median stem of which ends in the upper lip, gives off four branches in its course: the common trunk of the ophthalmic arteries, the cerebral artery, and the two antennal arteries. It may be of interest to notice that the sternal artery in Schizopoda arises from the heart.

The structure of the aortic valves themselves is different from those of Arachnida. They are paired lateral structures, as in all other Crustacea. The position indicated by Delage, taking into account evidence derived from the study of all other organs, is two somites nearer the head than in Arachnida. In such Decapoda as the crayfish and the lobster the heart is distinctly limited and considerably modified. Instead of arising from the aorta, the antennal arteries arise directly from the heart and have their own valves. Yet the aorta has also valves at its base and these are of the same type as in Schizopoda and Amphipoda. The position of these valves coincides exactly with the semilunar sulcus of the carapace, the two ends of which open into the so-called cervical groove. There are therefore differences in regard to structure of the circulatory system in closely related orders of the same sub-class—differences which cannot be understood without special study directed to their elucidation.

We know still less of the Protracheata, Pycnogonida, and the four classes formerly comprised under the general name of Myriapoda. Although I have some investigations under way, I am not prepared as yet to make any definite statement.

The circulatory system of insects is somewhat better known in this respect, yet here also the data are quite inadequate to form a clear judgement. Popovici-Baznosanu has described the heart in the Chironomus larva and states that the aortic valves are situated close to the anterior end of the fifth segment. In other larvae the heart had been described by other authors as situated near the rear end of the body. In some larvae the heart is not even situated directly under the dorsal body wall, but lies considerably deeper in the body cavity. The structure of the cardio-aortic valves, too, seems to be not only different from that of the aortic valve in Arachnida, but not always of the same type in all insects. Moreover, according to Zawarzin there are modified ostia in the aorta itself. It is evident that the first step must be in finding the true limits of the heart itself in insects. Meanwhile all conjectures would be entirely out of place.

SUMMARY AND CONCLUSIONS

1. In comparing the segmentation in arthropods the uncertain method of counting somites beginning with the anterior end should be abandoned. Instead, some structure should be chosen which has permanent value for a number of forms within a class and used as a starting-point of comparison.

2. Such structure in the case of Arachnida is furnished by the cardio-aortic valve which marks the division line between the last thoracic and first abdominal segments.

3. The method applied to Limulus leads to the conclusion that the carapace of Limulus is more complicated than in Arachnida, having two abdominal tergites drawn into the horseshoe-shaped thoracic tergite with which they have fused anteriorly and laterally.

4. A further conclusion is that the midcorporal articulation in Limulus is not between thorax and abdomen, but between the second and third abdominal somites.

5. The structure of the circulatory system in Arachnida follows a general plan given in the text.

6. The number of postoral somites in adult Archnida is seventeen. Five of these are thoracic and twelve abdominal.

7. The genital opening is on the second abdominal somite.

8. The first caudal segment in scorpions is not a true somite, but merely the anterior division of the fourteenth postoral somite.

9. If the number of preoral somites in Arachnida is not more than four, as represented by the median ocular, lateral ocular, rostral, and cheliceral somites, then the total number of somites in Arachnida is twenty-one.

EXPLANATION OF PLATES

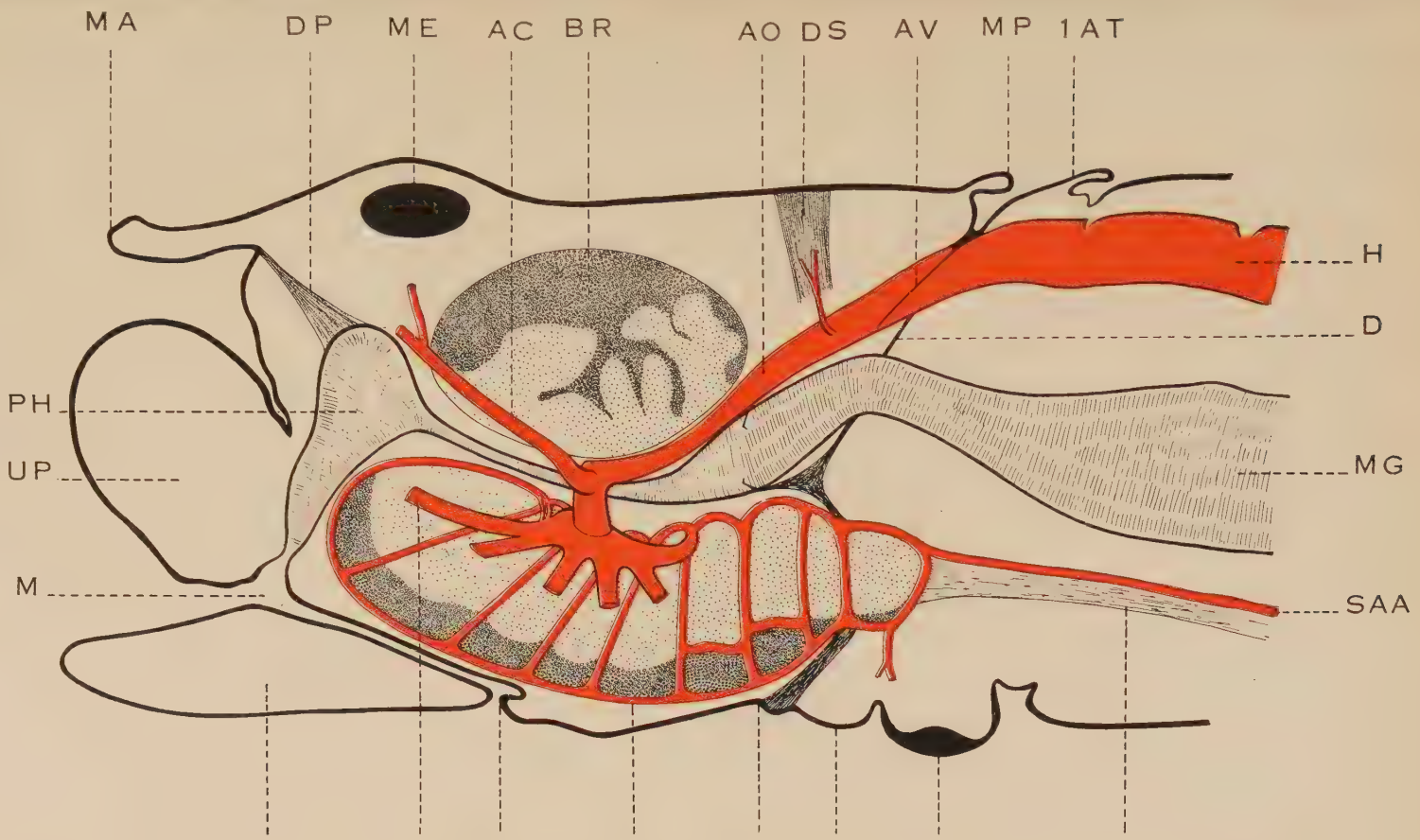
ABBREVIATIONS

AC, Cheliceral artery
AO, Aorta
AP, Pedal artery
APP, Pedipalpal artery
1. AT, First abdominal tergite
AV, Anterior aortic valve
BR, Brain
CA, Comb-artery (ninth vertical artery)
Cbp, Basal plate of comb
CO, Aortic arch connection with thoracic sinus
D, Diaphragm
DP, Dilator muscle of the pharynx
DS, Dorsoventral muscle separating the first cephalothoracic diverticle of the midgut from the second
GB. 2, Gnathobase of the second leg
GP, Genital plate (operculum)
H, Heart
LC, Longitudinal connective between the fourth and fifth abdominal ganglia
M, Mouth
MA, Anterior edge of carapace
ME, Median eyes
MG, Midgut
MP, Posterior edge of carapace
PH, Pharynx
SAA, Supraneural (epineural) artery
SBA, Subneural (hyponeural) artery
ST, Sternum
UP, Upper lip (rostrum)
VA, Vertical or interneural artery

PLATE 1

EXPLANATION OF FIGURE

1 The circulatory system of a young scorpion, Centrurus insulanus. A very perfect median sagittal section, 20μ thick, was first drawn with the Edinger drawing apparatus. The section contained everything shown in the figure except the cephalic arteries, the thoracic sinus and the arteries leading to the appendages. All these were introduced into the drawing on the basis of results obtained from the study of a series of frontal sections through scorpions of the same age and species and of adult injected specimens of Centrurus carolinianus from Texas. To avoid confusion, the corresponding arteries of the right side are omitted. The cephalothoracic diverticula of the midgut are also omitted. In the section from which the figure was made they extend as far as the median eyes.


MA
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GP
CBP
LC

FIG 1

PLATE 2

EXPLANATION OF FIGURES

2 The circulatory system of a young scorpion, Centrurus insulanus. Dorsal view of the thoracic sinus with its arteries and of the supraneural artery. This figure represents a combination drawing of three consecutive sections of a frontal series. The drawing was made with the Edinger drawing apparatus under the same magnification as figure 1. Heart, aorta, cephalic arteries and subneural artery are not shown because they belong to quite different levels. The place where the aortic arch connects with the thoracic sinus of the corresponding side is indicated by the black oval opposite the base of the second pedal artery. Similarly, the vertical arteries connecting the supraneural with the subneural artery are represented by small black circles. The dotted parts represent the nervous system.

3 One of the right ostia in the heart of a young scorpion, Centrurus insulanus, drawn from a frontal section under high power. Between the fibers of the pteripyle ligament blood corpuscles are shown.

4 Transverse section through the aorta of a young scorpion, Centrurus insulanus, showing at a high power the aortic valve.

5 Comb-artery of a young scorpion, Centrurus insulanus, drawn from a thick transverse section. The drawing shows the fourth abdominal ganglion and the genital duct with its muscles.

6 Sagittal section through the heart of a young spider, Lycosa carolinensis, in the region of the aortic valve. The drawing shows the relation of the valve to the carapace for comparison with figure 1.

7 Transverse section through the aorta of a young spider, Lycosa carolinensis, showing the aortic valve, for comparison with figure 4.

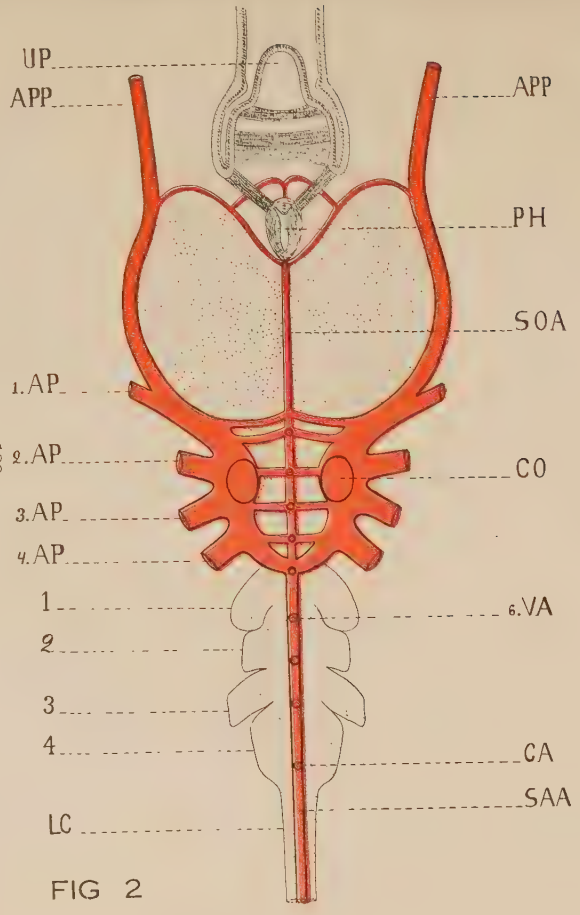

FIG 2

FIG 3

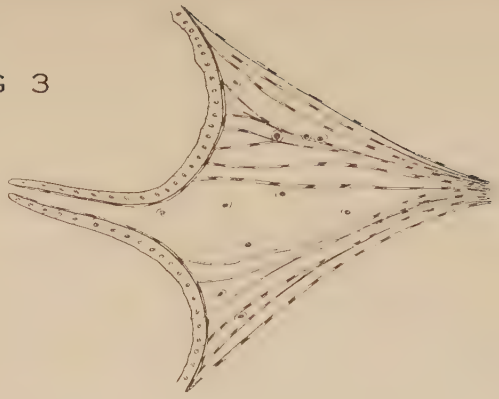

FIG 4

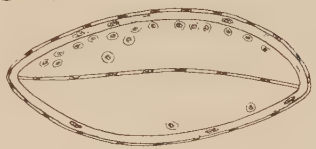

FIG 5

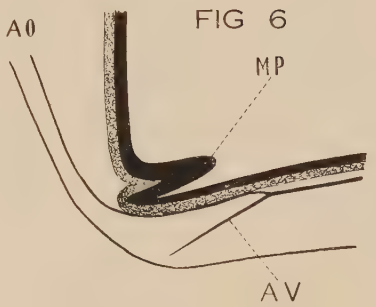

FIG 6

FIG 7

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1922, xix, pp. 290–291.

128 (1875)

On the method of macronuclear disintegration during endomixis in Paramecium aurelia.

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In the original studies[1] of the nuclear phenomena involved in endomixis, it was found that macronuclear disintegration, both in *Paramecium aurelia* and *Paramecium caudatum*, was effected by the elimination of spherical chromatin-bodies from the macronucleus, instead of by the transformation of most of the macronucleus into long tangled chromatin-ribbons such as occurs during conjugation in these species. Regarding *Paramecium aurelia*, it was stated that the "differences between the macronuclear changes during conjugation and during the process (endomixis) are only morphological; on the one hand, the macronucleus forms 'wurstförmige Schlingen,' while on the other, the macronucleus eliminates its chromatin by extruding it in the form of spherical bodies." [2]

This contrast proved valid not only in Woodruff's pedigree race[3] (I) of *Paramecium aurelia* in which endomixis was discovered, but also in animals from such diverse sources as Germany and Ohio. Only one cell (*Paramecium aurelia*, I), 4087th generation, December 6, 1913) was observed during endomixis which in any way even suggested ribbon formation and a figure was given of this animal with the legend "An atypical form of macronuclear disintegration, slightly resembling the ribbon-like formation characteristic of conjugation." [4]

It is therefore interesting to record that the study of animals from this same pedigree culture (I) of *Paramecium aurelia* at about

[1] L. L. Woodruff and Rhoda Erdmann, *Journ. Exper. Zoölogy*, 1914, xvii, 425–517. Erdmann and Woodruff, *Journ. Exper. Zoölogy*, 1916, xx, 59–97.

[2] Woodruff and Erdmann, loc. cit., pp. 438–39, and 444; Plate 1, Figs. 9 and 11; Plate 2, Fig. 14; Plate 3, Fig. 32.

[3] Woodruff, *Biological Bulletin*, 1917, xxx, 51–56.

[4] Woodruff and Erdmann, loc. cit., Plate 4, Fig. 37.

the 8900th generation (November, 1921) showed some cells successfully undergoing endomixis with macronuclear disintegration by ribbon formation and others by chromatin-body formation.

Thus it is clear that although all the data thus far at hand indicate that chromatin-body formation is the typical method of destroying the macronucleus in endomixis, nevertheless under certain unknown conditions the formation of chromatin-ribbons, until now regarded as diagnostic of conjugation, occurs in endomixis. This fact, of course, in nowise narrows the significant and crucial difference between endomixis and conjugation—the absence of synkaryon formation in the former and its presence in the latter.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, MARCH 20

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THE REACTIONS OF AMBLYSTOMA TIGRINUM TO OLFACTORY STIMULI[1]

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ONE FIGURE

INTRODUCTION

The existence of the sense of smell in fishes has been clearly shown by Parker, Sheldon, and others. This work has settled the question for this group in accordance with Sherrington's definition, which places the sense of smell in the exteroceptive system and that of taste in the interoceptive system.

Comparatively little work of an exact nature has been done upon the olfactory sense of amphibia. Reese ('12) tested Diemyctilus with food and meat juices, but as he failed to control the sense of sight, his results are not fully conclusive.

Copeland ('13) repeated the experiments of Reese, using more exact methods. He controlled the visual sense by stimulating the olfactory epithelium with diffusion from a motionless source, and he concludes that there is a definite olfactory sense in this animal.

Risser ('14), working upon larvae and adults of the frog and the toad, showed that tadpoles of the toad possess an olfactory sense, while frog tadpoles do not. In the adult toad, he was unable to obtain satisfactory data in regard to the existence of an olfactory sense except in regard to specific odors. He did not, however, obtain any response to food substances with which the animals normally come in contact.

Burr ('16) obtained definite olfactory responses in larval Amblystoma punctatum.

An investigation was begun in the spring of 1919 in an endeavor to study the correlation of the senses of sight and smell after

[1] Anat. Rec., vol. 20, 1921, p. 189.

extirpation of the sense organs in the larvae. Unfortunately, all of the animals so treated failed to survive. The present work was started in the fall of 1919 in order to get control results for the data which it is hoped can later be obtained upon adult forms which have been operated upon in early stages of development.

I take pleasure in thanking Dr. Henry Laurens, under whose direction the work has been carried on, for many helpful suggestions and much useful criticism. The general observations upon the behavior of the operated larvae here described were made by Doctor Laurens, who performed the necessary operations in the spring of 1917.[2]

MATERIAL AND METHODS

The same general method of approach was used as in Burr's ('16) work, i.e., removing the embryonic anlage of the sense organs. Since the method of operation has been described in earlier papers (Laurens, '14, and Burr, '16), no description will be necessary here. The operated larvae were kept in battery jars stocked with the small crustacea, Daphnia and Cypris, and were allowed to grow to a length of about 25 mm. before any of the observations were made.

The adult animals, which were used for the main portion of the work, were three individuals selected from a number that had been sent to Doctor Laurens from Albuquerque, New Mexico, in the spring of 1919. The same individuals were used under all the different conditions described in the experimental section, in order that the general control of the reactions might be more accurate and a more reliable average of reaction obtained.

The methods applied to these forms were mainly those which have been used by Parker in his work on Fundulus and Ameiurus, with an application of some of Risser's methods of experimentation. In all the experiments the test aquarium was cleansed after each operation and filled with fresh water before continuing the tests.

[2] The larvae on which the general observations were made were reared from eggs sent to Doctor Laurens by Prof. C. P. Sigerfoos, in the spring of 1917. Dr. W. C. Allee also was kind enough to send the author the eggs of A. tigrinum, but unfortunately, owing to the vicissitudes of the journey and the shock of subsequent operation, these animals did not survive.

NORMAL BEHAVIOR

The larvae are in almost incessant motion under laboratory conditions, swimming about the aquaria apparently in a constant search for food. Only after they have become gorged with food will they sink to the bottom, and even then they are seldom motionless, but continue to move about by combined walking and swimming movements, stopping to examine any object that attracts their attention.

The adults used in this work had lived under laboratory conditions for three months previous to the time of the experimental work. The movements of the adult animals are ordinarily not particularly rapid, but the application of stimuli causes a remarkable acceleration of reaction. If placed in an aquarium provided with a float and left undisturbed, the animals will generally be found perfectly motionless upon the float, or else partly submerged with the head protruding above the surface. When placed in an aquarium filled with water to a depth of 6 or 7 inches, but without a float, the animals are restless for a time after their introduction, due probably to the handling which they have received. They soon come to rest, however, with only an occasional sluggish movement. The animals were admirable for the experimental conditions imposed. They can be handled singly and show no trace of the educative process which is said to occur in some forms, viz., Ameiurus, after having been subjected to trials of this nature. An attempt was made to obtain evidence of any difference in reaction which might occur in this way by taking the speed of the reaction as an indicator. The last control experiments, with the conditions as nearly as possible like the original experiment, showed an average time slightly longer than the original.

During the process of acclimatization, the animals were fed solely upon small pieces of earthworm. The degree of visual sensibility is high. In many cases the animals will secure the earthworm while it is sinking to the bottom of the aquarium. If the food has come to rest, the animals often take great interest in the observer, and for this reason the walls of the aquarium

must be screened for accurate observation in experimental work. The screening has the added advantage of reducing the amount of light in the aquarium and rendering the animals more quiet than they are under a light of higher intensity. The animals have a slight tendency to be more restless than usual under conditions of hunger.

RESPIRATORY MECHANISM

Bruner ('14a) has described the mechanism of the olfactory sense organ in relation to the respiratory mechanism in a comparative study of a number of Amphibia. He states that in Amblystoma larvae the olfactory process is similar to that of frog tadpoles. "Respiratory water is taken through the nostrils and is the only medium of smell." He finds that the passage from the mouth to the nasal cavity is occluded by a fold of mucous membrane, the choanal valve, which prevents either air or water from passing out in this direction. These choanal valves persist until metamorphosis. He distinguishes in general two distinct types of respiratory mechanism: in one the respiratory medium is allowed to pass from the nasal cavity to the buccal cavity, the passage of return being occluded by the choanal valves; in the other the respiratory mechanism is completely under muscular control and the respiratory medium can pass freely in and out of the nasal cavity. According to the type of respiratory mechanism present, he classifies the forms as "monosmatic, single smellers, in which the olfactory organ is used to test only the external medium; and diosmatic forms, double smellers, including Siren, Cryptobranchus, Amphiuma, larvae of lungless salamanders and the adult stage of higher amphibians." In the diosmatic forms, the olfactory organ is used in testing the content of the buccal cavity as well as that of the external medium.

Bruner ('14b) mentions that Jacobson's organ is present in Amblystoma adults and that, from the existence of this organ, this form should be classed as a diosmatic form.

As a preliminary to the experiments on the olfactory sense, it was deemed expedient to make a study of the water currents

passing through the respiratory mechanism and their direction and disposal.

Blinded adult Amblystoma were used on account of the ease with which they can be handled as well as the fact that they show less reaction to the light stimulus occurring under the conditions necessary for observation.

The animals were placed in a flat glass dish under the binocular microscope and the water currents observed by means of a carmine suspension. Three different courses of these currents have been observed. 1) A current taken in through the nasal openings and expelled through the mouth. This condition is the customary one persisting during the state of rest of the animal. 2) A current entering through the nasal passage and the mouth and expelled through the same channels. This is the condition occurring under stimulation, both the incurrent and the excurrent phases taking place with a quick spurt. 3) A current of water expelled through the nasal passages (this being the discharge of the content of the buccal cavity), a special case occurring only when the animals comes to the surface to obtain 'gulps' of air. Air is taken in either through the nose or the mouth. The latter is used for the quick 'gulps' of air which the animal secures as it appears momentarily upon the surface, while the nasal apertures are used when the animal is in a state of rest, lying in the water with the snout protruded above the surface. The current designated above as 3 occurs infrequently as the aftermath of the second method. As a stimulus becomes attenuated by diffusion, the animal seems to take in water more quickly through the mouth that it can be discharged. At such a time the currents seem to be simultaneously going into the mouth and out through the nasal passages.[3]

[3] Vincent and Cameron find that respiratory movements cease in the frog as soon as the nares of the animal are under water. This condition has been observed by them over several weeks. A preliminary set of experiments, in which normal, anaesthetized and decerebrate frogs were used, shows that the inhibition of the respiratory movements is temporary and that water currents can be detected by means of the method given above. The efficiency of the valves covering the nares decreases with the time of immersion. None of the animals used in this work survived twenty-four hours of immersion.

EXPERIMENTS ON LARVAE

Four series of larvae were kept under observation, namely, 1) eyeless, those animals in which the optic vesicles had been removed; 2) noseless, those which had been deprived of the nasal placodes; 3) eyeless and noseless, those from which both the optic vesicle and the nasal placodes had been removed; 4) a series of normal animals which served as controls and were of considerable use in a comparative study.

Under practically the same conditions, it could be easily seen that the normal larvae soon outdistanced the operated in growth. The noseless animals were slightly smaller than the normals of the same age. Next in size were the eyeless forms, and finally the eyeless and noseless, which were considerably smaller than the eyeless.

In a general way, the rate of growth shows the relative importance of the senses of sight and smell. The sense of sight is of more use in the obtaining of food at this stage than is that of smell. If the conditions of the amount of food present are varied, however, there is a change in the relative importance of the senses, for when food is scarce the sense of smell seems to dominate. This is shown in the following observation. The noseless forms seem to have no ability for distinguishing whether a particle is a food substance unless that particle has motion. The eyeless forms have a distinct advantage in this connection for they will distinguish pieces of organic material. If pieces of dead earthworm are placed in an aquarium, the noseless animals will push them around as they do other bits of debris, but will seldom snap at them. The eyeless, in direct contrast to this, will devour the earthworm immediately after coming in contact with it.

A marked difference was noted in the responses to rapidly diffusing food substances obtained in the different groups of the larvae. When freshly cut earthworms are placed in the aquaria, the normal animals begin to swim rapidly about without making any attempt to locate the stimulus. They will often snap as they pass through the area occupied by the diffusing substances.

but keep swimming on and do not stop at the point of the diffusion which would supposedly be, in this case, the center of attraction. This stimulus is not sufficiently definite and the animal swims about until attracted (by sight) to the motion of the piece of worm, which is then seized.

The same indications are given with beef juice or with strips of beef instead of earthworm. The animals are stimulated by the diffusion, but seldom engulf the food if it remains motionless. When the strip of beef is moved by means of an attached thread, the animal is quickly attracted and speedily engulfs it.

The eyeless forms, under the same conditions, also show signs of stimulation. Their ability to locate food is less than that of the normal larvae. They are easily attracted to any slightly moving object, and since they lack the sense of sight it must be concluded that there is another factor entering into the reaction, that of sensitivity to vibration stimuli. Under conditions of starvation, an increase in sensitivity to the diffusion of passive or motionless objects must result, for the eyeless larvae will locate and devour the fecal material within the aquaria.

The noseless forms are not stimulated by diffusing substances. They swim passively about the aquaria after the introduction of food materials of different sorts and unless the food exhibits motion it is entirely overlooked. Pieces of beef are completely ignored, producing no modification in the behavior of the animal unless they are agitated, when they are quickly engulfed.

These experiments, made on larvae of Amblystoma tigrinum, thus confirm the results of Burr ('16), who used A. punctatum. The work has been further extended by the study of individuals deprived of both eyes and nose.

The eyeless and noseless forms show the same behavior as the noseless. In general, these larvae remain quiet in the aquaria and move about only at intervals. They are absolutely dependent upon mechanical stimuli in finding food. This is clearly shown by the way in which they ignore organic material such as killed earthworm or bits of beef. Under conditions of starvation, they exhibit a certain foraging reaction, pushing the objects about within the aquarium in order to discover whether inherent

motion is present in these objects. If such motion occurs, the animal is stimulated mechanically. At times, however, these animals will snap at any object with which they may come in contact and engulf it with a subsequent elimination of the substances unfit for food.

In addition to the observations described above, several experiments were made using balls of filter-paper which had either been previously treated with juices of beef or earthworm or had been left untreated. A few experiments with sacks containing test substances were also made. As the result of these tests it may be said that there is a strong indication that the eyeless larvae distinguish their food substances by the sense of smell and mechanical stimulation. The normal animals are mainly dependent upon sight for the final location of food. The noseless larvae are dependent upon visible motion. The noseless and eyeless larvae have only mechanical stimuli to guide them.

EXPERIMENTS UPON THE ADULTS

1. The reactions of normal animals in the light

Reactions to motionless objects. The method used in this case was the same as that used by Parker and Sheldon in their experiments with Ameiurus, Mustelus, and Fundulus.

An individual was placed in an aquarium, the sides of which had been screened to obviate any distractions that might occur from the presence of the observer. The aquarium was a glass-enclosed one, 42 cm. in length and 26 cm. wide. This was filled to a depth of 10 cm. with water. After allowing a period of ten minutes for the animal to become adapted to the test aquarium, small cheesecloth bags, equal in size and suspended at the same height in the water, were introduced. These bags allowed a rapid diffusion of any substances that might be contained within them. The bags were hung from a rod at the top of the aquarium by means of a cotton thread. The time taken for the introduction of the test materials was at that period at which the animal had come to rest at some position near the central portion of the aquarium. The bags were then introduced with as little disturbance of the medium as possible. Because of the variation of

the conditions under which experimentation took place, the stimulus caused by the introduction of the test sacks is negligible. One of the bags was filled with minced earthworm and was weighted with sand, while the other bag contained sand alone. Ten trials were made in each series, the three animals being tested in turn with at least a thirty-minute interval between trials.

Under normal conditions, the adult Amblystoma moves slowly about the aquarium seeking for a projection upon which it can rest, preferably upon the surface of the water. Under conditions of stimulation the animal becomes restless, hastening its ordinary movements and proceeding with a greater degree of rapidity about the aquarium. It is this increase in activity that is spoken of in the following series as the 'beginning reaction.' All reactions in this series were considered to be consummated when the testing material was seized by the animal.

The conditions under which each animal was tested were constant in regard to the motion of the test substances. The other factors, however, such as the distance of the test substance from the animal, the distance of the test substances from each other, and the position of the test substances with reference to the animal were considered. The variations in the conditions preclude any factor that might arise in connection with the above.

The response to the stimulus was, in every case, a positive one. Differentiation between the test substances was always in favor of that sack containing the food material. In order to show a few of the experiments and the variations imposed as well as the procedure, two of the individual cases are given below.

Trial 4. Animal A. Placed in aquarium at 11.05. Animal is restless. Test sacks introduced at 11.20. The animal moves restlessly about sides of aquarium, moving to center of the aquarium where the sacks are suspended within 2 inches of each other, noses earthworm bag, circles sand bag, goes to earthworm bag, noses, snaps, and seizes. Reaction is completed in two minutes.

Trial 7. Animal A. Placed in aquarium at 3.10. Test sacks introduced 3.15. The animal swims about, passes sand bag,

approaches earthworm bag, and seizes. The animal was detached from bag as quickly as possible and sand bag substituted for it. Animal pays no attention to this bag after nosing it. Meanwhile earthworm bag had been placed at far end of aquarium. Animal turns quickly after nosing sand bag, swims to far end of aquarium, noses bag and seizes. The first reaction is complete in three minutes, the second, seven minutes after the introduction of the sacks.

The results of this series are tabulated in table 1, series 1 (a), at the end of this section.

Reactions to moving objects. The same kinds of testing sacks were used, the conditions of the trials being duplicated in so far as possible with but a single variation, that of a moving object. A rod, from which the testing sacks were suspended, was held by the observer. The time element is partially lost in this case, and because of the varying distances of stimulation due to the motion of the test sacks, it is deemed an unimportant factor in this connection. It is included in the description of the individual trials, in order to show the difference in the complete action time for the trials under consideration. The reaction is considered positive when the test sack is seized or an attempt is made to seize it.

The record of one individual case will be given here as typical of the series. The results are tabulated in table 1, series 1 (b).

Trial 1. Animal C. Placed in aquarium at 10.50. Sand bag, introduced at 11.00, kept in motion in front of the animal, but held at short intervals at different parts of the body. Animal turns at the first approach of the bag and noses it, after which it paid no attention to it, although it was kept moving about the animal for five minutes. Earthworm bag was substituted for sand bag after sand bag has been drawn to end of aquarium away from animal. Earthworm bag played before animal. Animal follows bag about aquarium, noses it when it comes to rest and then seizes. Sand bag substituted and animal allowed to nose it, does not follow it, but moves away, coming to rest at opposite side of aquarium.

All the experiments were positive in this series, the animal showing a distinct discrimination between the test substances.

2. Terrarium experiments

Reactions to motionless objects. A few experiments were made with the animals in a terrarium. The same substances were used as in the preceding trials. The animals are almost insensible to the presence of test substances, and in but one trial in ten was there any indication of a detection of the difference between the substances contained in the bags. This was the only reaction given, all other trials giving no reaction to either bag. The procedure followed was simply to place the bags near each other at the bottom of the terrarium and await the reaction. The one case recorded as positive showed a reaction about fifteen minutes after the introduction of the materials, the animal nosing the earthworm bag after passing the sand bag, and snapping the earthworm bag.

A comparison between the sensitivity of the adult animal in the air and in the water can easily be obtained in the ordinary process of feeding. Cut earthworm can be held against the nasal apertures of the animal and elicit no response. If, however, the animal is so situated that its nasal openings are immersed in water, a very small diffusion from the forceps in which earthworms have been held is sufficient to give an extremely active response.

Reactions to moving objects. If the test sacks are moved about in a terrarium in the same fashion as that described above for the animals in the water, a decided reaction is obtained. In many cases the animal seizes the bag with such force that it can be lifted from the bottom. This is particularly true with reference to the earthworm bag, for the animal will quickly relinquish the sand bag. It is probable that the basis of discrimination in this case is one of taste. It is likely that this is the same reaction as that obtained in the noseless larvae, when the sense of taste or perhaps of touch, must enter into the reaction of the rejection of particles unfit for food.

3. The reactions of normal animals in the dark room. (In aquarium)

The animals were dark adapted for two days before the experiments were undertaken and were given no food for three days. After dark adaptation, the animals are noticeably more sensitive to handling and the time required to come to rest is lengthened appreciably. A dim red light of minimum intensity, just sufficient for the observer, after a dark adaptation of forty minutes, to see the animal in profile, was used for the observations. The intensity was so weak that the figures upon a watch dial could be read with difficulty just in front of the screened light source. This degree of light is probably below the visual threshold of Amblystoma (Arey, '19), as evidenced in the reactions of the animals during the period of observation preceding the actual experiments. Ordinarily, with one side of the aquarium more brightly illuminated than the other, the animal, particularly if hungry, will tend to move about the sides of the aquarium with a preference for the brighter side, being often attracted to its own reflection in the glass wall, as well as by slight visible movements at the side of the aquarium. Under the dark condition, the animal moves slowly up and down until it is stopped by the aquarium wall, then turning, it may proceed down the center of the aquarium, showing no particular preference for the wall.

Reactions to motionless objects. The test bags were used as in the preceding section and the description of one individual case will show the procedure with the unvarying positive result (table 1, 3a).

Trial 1. Animal B. Tested by the offering of the two test sacks. The earth worm bag and the sand bag were placed at opposite ends of the aquarium. The animal shows the characteristic motor reaction soon after the introduction of the material, but seems unable to locate it. Finally locates the object. Positions of bags reversed and operation repeated. Animal swims restlessly about for some time before approaching object, noses it, and seizes it. As an additional test, a glass vial containing freshly cut earthworms was placed in the aquarium. The animal immediately becomes restless and, after swimming about for

short while, makes for the glass tube, approaching it from closed end which was purposely placed toward center of aquarium while open end of tube was situated toward side of aquarium and touching it. After nosing tube, animal follows up side of tube to get to open end, pushing tube across aquarium in an effort to obtain diffusing contents. An empty vial produced no reaction.

Throughout this series of experiments, the reaction to the vial filled with earthworms was much faster than that given to the test sacks. The amount of diffusion is greater in the vial experiments and the diffusion takes place from one side of a definite focus in contrast to the test sacks which diffuse on all sides. It is likely that the strength or concentration of the diffusing substances plays a large part in the difference of the speed of the two reactions.

Reactions to moving objects. The animals were subjected to tests with moving objects as in the preceding section. One typical case will suffice to show the manipulation used and the general result.

Trial 1. Animal B. Sand bag twitched gently in front of animal. Animal follows bag as it is drawn across the aquarium. Animal noses bag when it comes to rest. No further reaction. Trial repeated with same result. Earthworm bag twitched before animal. Response immediate, animal follows, noses when at rest and seizes.

The reaction time of normal animals in darkness is much slower than the reaction time in the light, but none the less definite. The sensitivity of the animal to vibration stimuli seems to be increased, as shown by the fact that the animal will follow the sand bag although moved very gently so that the amount of motion given to the surrounding medium is quite small.

4. Experiments upon nose-stopped animals

Many methods for occluding the sense of smell have been tried by experimenters. The closure of the external nares by stitching has been used by Parker and his coworkers. The cotton-wool plug used by Sheldon and later by Parker has been

satisfactory on the forms used by them. In some cases the olfactory tracts have been cut. The latter method is the most effective, but has the one disadvantage of not permitting a return to the normal for control experiments. The cotton-wool plug has been found to have certain irritating effects upon the nasal epithelium and for this reason as well as for ease of handling, a collodion mask was used for occluding the nasal passages in this work.

The method is quite simple. The animal is removed from the aquarium and wrapped in a small hand towel with only the head projecting. The anterior portion of the head is dried as much as is possible and the collodion applied over the surface prepared in this way and across the nares, making a mask the shape of an inverted T, the base of the T extending well up between the eyes, while the cross line covers the external nares and extends well around the maxilla. The mixture dries rapidly and during drying causes some irritation. The animal will get rid of the cap if its forefeet are free at this stage. The cap will sometimes be removed by the force of the water from the mouth or by an excess of mucous secretion on the front of the head, but after three or four applications the animal becomes accustomed to the mask and shows little or no sign of irritation. At this stage the nose cap is more or less firmly adherent to the head of the animal. The animal is replaced in the water of the aquarium and generally becomes quiet in ten or fifteen minutes. If the cap has remained fast during this period, it will probably remain fixed for at least twelve hours, during which time experimentation may proceed.

The respiratory process of the animal is changed by this occlusion of the nares. The animal comes to the surface and 'gulps' air by means of the mouth, then sinks and exhales slowly, the bubbles of air escaping at the mouth. The act of respiration affords a constant check upon the effectiveness of the nose mask, for if ineffective the water current under pressure from the buccal cavity will soon remove it. This mask is easily and quickly removed from the animals, after which they respond to control tests in exactly the same fashion as in the normal

experiments. For the purposes in hand, the method was admirable, although it did require a great deal of patience and considerable observation to make sure that the nose caps were tight. It was with the idea of making a combination hoodwink and nose cap that this method was applied, but the irritation resulting from the application of the mixture of lampblack and collodion to the eyes of the animal was too great to get normal reactions.

Reactions to motionless objects. After securing a nose mask which was satisfactory, the animals were placed in screened aquaria in order that the observer might be concealed from view. The reaction time was much increased. Twelve minutes, three times as long as the average reaction time for the normal, was allowed before a test was pronounced negative. The reactions were recorded as negative when the animal made no effort to obtain either bag and also showed none of the characteristic motor activity of a stimulated animal, as positive when the animal attempted to nose either bag. In the reactions so recorded, this reaction occurred within five to seven minutes after the introduction of test materials into the aquaria. After the attempt to nose, the animal paid no further attention to either of the bags.

The reactions obtained show distinctly that animals with the sense of smell occluded cannot discriminate between test substances, for there was in no case any preference shown, the animals nosing the sand bag, on the average, just as frequently as the earthworm bag. The reaction is, to a large extent, one of contact and is accidental. (Table 1, series 4 (a).)

Reactions to moving objects. In contrast to the above reactions, the reactions of the nose-stopped animals to moving objects is quite rapid. The test sacks were moved about the aquarium at various distances from the animal. All the reactions gave an average reaction time of two and two-tenths minutes, the animals showing no discriminaton between the test substances, in marked contrast to the selective reaction obtained from animals with the possession of the normal respiratory mechanism. (Table 1, series 4 (b).)

5. Experiments with blinded animals

The animals were anaesthetized with chloretone and the eyes removed with a pair of fine scissors. After several hours the animals seemed normal and were fed. They were then not fed for a period of five days preceding the commencement of the following experiments. Animal A, which was not normal, due to an inflammation of the external nares, was not used in the course of these experiments. The tests were made with the usual test sacks. Results are tabulated in table 1, series 5 (a) and (b).

The average reaction time in this series is almost the same as that for the normal. All the tests showed that the animals discriminated between the test substances, reacting positively to the earthworm sack.

In the reactions to moving objects, ten trials were made, all giving the same end result. The animals were quickly attracted by any movement in the water. They would not, however, follow the sand bag, although stimulation was shown by their motor activity. They did follow the earthworm bag and would, if possible, snap and seize it.

6. Experiments with nose-stopped and blinded animals

The external nares of the blinded animals were covered with collodion and the animals were subjected to the same trials as those given above. The results are the same for both the moving and the motionless objects. There was no attraction to either object in the motionless series. In the series with the moving object, there was a slight motor activity present but no definite objective. The results are tabulated in table 1, series 6 (a) and (b).

7. Partitioned aquarium experiments

An aquarium was divided into two compartments by the insertion of a glass partition which ran diagonally across it, having a slit three-eighths of an inch high between the base of the partition and the concrete bottom of the aquarium. This slit afforded the means by which diffusing substances could pass to the test

side. The arrangement proved very useful in the differentiation of sight stimulus from that of smell and was used on all of the four series of animals. Two kinds of substances were used, diffusible and non-diffusible but visible. For the first, an infusion was made of minced earthworm, generally allowed to stand over night and filtered in the morning. The resulting extract contained no visible solid substance, but simply the juices and body fluids of the earthworms. This proved to be a very potent sort

TABLE 1

Reactions to test sacks

SERIES		NUMBER OF TRIALS		CONDITION OF TEST SUBSTANCE	REACTION	TIME
						minutes
Normal	1	10	Aquarium	(a)Motionless	Positive	3.8
		10		(b)Moving	Positive	
	2	10	Terrarium	(a)Motionless	Positive	15
		10		(b)Moving	Positive	
	3	10	Dark room	(a)Motionless	Positive	Over 30
		10	In aquarium	(b)Moving	Positive	
Nose-stopped	4	10	Aquarium	(a)Motionless	Attracted	12
		10		(b)Moving	equally	
Blinded	5	10	Aquarium	(a)Motionless	Positive	4.2
		10	Aquarium	(b)Moving	Positive	
Nose-stopped and blinded	6	10	Aquarium	(a)Motionless	Negative	
		10	Aquarium	(b)Moving	Negative	

of stimulus. It was applied by means of a pipette along the wall of the aquarium, and diffusing through the water passed under the partition. The observer was screened from view. The reactions were remarkably quick and vigorous, in many cases the animal moved to the central portion of the partition and snapped at the diffusing substances which it perceived but certainly did not see.

The non-diffusing substances used were bits of rubber tubing and uncut earthworms. According to the work of Olmsted ('18),

uncut earthworms will stimulate Ameiurus by slime secreted by the skin. This is not the case in A. tigrinum, a fact which may indicate that the probable sensitivity of combined air and water breathers is not so great as single smellers. Red rubber tubing of small diameter was used to simulate earthworms. Uncut earthworms were allowed to move about in the half of the aquarium not containing the Amblystoma. The rubber tubing was more effective in eliciting a response if set in motion by means of

TABLE 2

Diffusing substances

TRIALS	SERIES	SUBSTANCE	REACTION
10	(1) Normal	Earthworm infusion	Positive
10	(2) Nose-stopped	Earthworm infusion	Negative
10	(3) Blinded	Earthworm infusion	Positive
10	(4) Blinded and nose-stopped	Earthworm infusion	Negative
Control, distilled water.....			Negative

TABLE 3

Non-diffusing substances

NUMBER OF TRIALS	SERIES	STIMULUS	REACTION
10	(1) Normal	Uncut earthworm and tubing	Positive
10	(2) Nose-stopped	Uncut earthworm and tubing	Positive
10	(3) Blinded	Uncut earthworm and tubing	Negative
10	(4) Nose-stopped and blinded	Uncut earthworm and tubing	Negative

a cotton thread. Both objects acted solely as visual stimuli, as can be seen from the accompanying table (table 3).

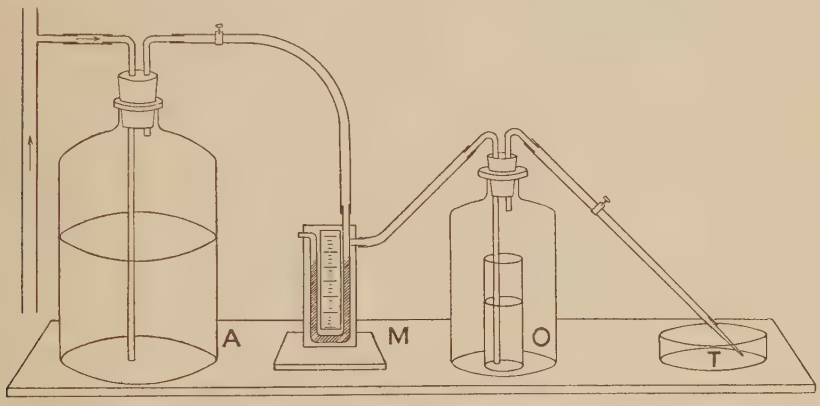
8. Experiments with odor streams

Risser has described an apparatus with which he tested the olfactory reactions of the toad. The apparatus consisted of a testing chamber into which odors were brought from an odor chamber by means of an air current.

A modification of this method was devised. The source of the air current was a compressed-air system, the air being run

through a bath of water in a 10-liter container. This container acted as a reservoir and served to keep the pressure constant (fig. 1).

The apparatus was first thoroughly tested for the effects of air currents under various pressures. It was found that an air current evidently stronger than that which Risser used had no effect upon the reactions of the experimental animal. This current could be distinctly felt on the lips or the surface of the tongue of the experimenter.



Text-figure 1 Diagram of the arrangement of the odor-stream apparatus From the flask (*A*) the air was run through a manometer (*M*), and from there to the odor chamber (*O*). A fluid, the odor of which was to be tested, was placed in a vial in the odor chamber and the air bubbled through the liquid. A glass nozzle, 0.5 mm. in diameter, was used to conduct the odor stream to the testing chamber (*T*).

For the experiments the Amblystoma were placed in a large dish, the bottom of which was covered with moist sand. Various pressures between 12 and 48 mm. of mercury were tried; temperature, 20°C. The average was 24 mm. of mercury. Air under this pressure emerging through a 0.5-mm. nozzle gives a displacement of 25 cc. of water per minute.

One of the individual experiments is here given in order to show the manipulation of the test substances as well as the behavior of the animal under the control trials.

Animal B: Control test with distilled water. Animal placed in testing chamber and allowed to come to rest. Air current

under pressure of 12 mm. of mercury applied to external nares, the nozzle being held at a distance of 1 mm. from animal. No muscular reaction and no respiratory reaction. Under a pressure of 36 mm., nozzle was held for three minutes with no reaction on part of animal except a raising of head. When air current was directed toward different parts of body (region behind the head, in front of the limb, trunk region, and the tail region) there was no reaction.

If the animals are partly submerged in water and the air current is allowed to produce a series of bubbles, the animal is

TABLE 4[1]

Reactions to odor streams

STIMULUS	PRESSURE	TIME	REACTION	TRIAL
	mm.			
Earthworm	24	3 min.	No reaction	Negative
Turpentine, oil of	24	3 min.	No reaction	Negative
Carbolic acid	24	3 min.	No reaction	Negative
Oil of cloves	24	20 sec.	Shakes head—draws away	Positive
Oil of wintergreen	24	3 min.	No reaction	Negative
Bergamot	24	3 min.	No reaction	Negative
Aniline	24	3 min.	No reaction	Negative
Ammonia, concentrated	24	1 sec.	Withdraws violently	Positive
Formol, 40 per cent	24	3 min.	No reaction	Negative
Chloroform	24	1 min.	Withdraws from current	Positive
Distilled water	24	3 min.	No reaction	Negative

[1] A trial is designated as negative when there is no reaction after three minutes' exposure; positive, when there is motor activity resulting from the stimulus.

mechanically stimulated and snaps at the bubbles. This stimulation is quite strong in the region just anterior to the forelimb, and is active, although less so, in the tail region. The trunk region proper shows but little sensitivity.

In testing odoriferous currents, a maximum exposure of three minutes was allowed, after which the trial was declared to be negative. The reaction was designated as positive if the animal moved toward the air current or away from it. A discussion of the results obtained and a comparison with those of Risser will be found in the next section.

DISCUSSION

The observations upon the operated larvae show that A. tigrinum possesses an olfactory sense. They also indicate the relative importance of the senses of sight and smell. Sight is undoubtedly the predominant sense in the obtaining of food, although it is rather interesting to observe that under conditions of paucity of food the animals in which the organ of sight has been removed, long before it could possibly function, have a more highly developed sensitivity toward objects giving an olfactory stimulus than do the normal animals. There seems to be a dominance of one sense over the other, this dominance being clearly detrimental to the animal under adverse conditions. This same condition is demonstrated in the observations upon the adult forms. A comparison between the ability of the blinded adults to obtain food and of normal individuals in the dark room is striking. Blinded adults give a positive reaction to olfactory stimulation in practically the same time as normal animals in the light. Risser has noted that there is what he has termed an inhibitory effect of the eyes over the nose in the toad. It is true that an animal possessing sight cannot accommodate itself to olfactory stimulation under conditions of darkness in the same time that blinded animals which have become accustomed to obtain food substances without the intermediation of sight can do so.

The factor of taste and its relation to the problem must be considered. Although the animals are nose-stopped (tables 1, series 4; 2, series 2; and 3, series 2), the organs of taste are undisturbed by this procedure. Water currents are passing in and out of the animal's mouth and some of the diffusing substance should come in contact with the taste buds. If the taste buds are responsible for the reaction, there should be a reaction even in nose-stopped animals. This is not found. In this case, however, the eye is a considerable factor, as is shown by the reaction elicited by moving objects. In the series with eyes removed and the nasal passages closed (tables 1, series 6; 2, series 4; and 3, series 4) there is no reaction to any stimulus applied. The sense of taste, therefore, can be regarded as a negligible factor.

The experiments in the partitioned aquarium (summarized in tables 2 and 3) show that animals possessing sight respond to stimuli, which because of their non-diffusibility have no effect upon the olfactory organ. The animals which have been deprived of sight can respond only to diffusing substances. In every case, the animals which could use neither sight nor smell are negative to all forms of olfactory stimulus.

Parker, in his work on fishes, found that the organs of the lateral line and the general chemical sense which are present in the skin had to be occluded in his analysis of results. This was accomplished by painting the animals with magnesium sulphate. This method was tried upon the Amblystoma adult to see if a definite factor of skin sensitivity might be present, but with no result. The use of alkalis or acids such as were employed by Parker and Sheldon ('12) or those used by Crozier ('16) might show the nature of this sense in the larvae of Amblystoma, but in relation to the stimuli employed, the skin sense was not shown to be in any sense discriminatory.

Parker and Sheldon ('12) point out that much of the work done by Aronsohn ('86) in which he uses oil of cloves in order to test the sense of smell in the goldfish, is inconclusive because of the use of an irritating substance. In their own experiments, they used normal substances for testing the olfactory sense.

The results obtained by Risser ('14) with the odor-stream apparatus are inconclusive. He states in his summary: "Odor streams specific in character, made to flow over and into the nasal openings stimulate the olfactory sense organ; such stimulation causing definite motor activities to follow," and further, "appropriate operation are confirmatory that stimulation by such odor streams is olfactory. Section of the olfactory tract inhibits the reactions. Olfactory stimulation and reaction are not affected by section of the ophthalmic branch of the trigeminal nerve."

The materials which Risser used and which, therefore, are to be assumed as odor streams 'specific' in character, are a large number of essential oils together with some food substances. A glance at his data shows that positive reactions were obtained

with irritating substances. The presence of substances such as ammonia or oil of cloves will cause a motor reaction in A. tigrinum as a reflex of skin irritation.

The results of the present series might be summed up as follows. Normal animals will discriminate between test substances and react positively toward the substance giving an olfactory stimulus, whether that substance is moving or motionless, and whether the animal is under conditions of light or of darkness.

Animals deprived of the olfactory sense by occluding the nasal passages show the same reaction to both test substances and react more quickly to moving substances than do the normal animals. There is no olfactory discrimination.

Animals which have been blinded respond to olfactory stimulus. If a moving object does not give rise to olfactory stimulation, the animal moves about in an indefinite manner without a definite objective, but will locate and try to obtain a moving focus giving an olfactory stimulus.

Animals which have been blinded and nose-stopped give no reactions except to moving objects and show no discrimination between test substances.

Normal animals give no reactions to normal substances carried in odor streams. They will, however, give motor reactions to irritating substances.

SUMMARY

1. General observations upon the growth and behavior of operated larvae show that the visual sense is the primary sense used in obtaining food.

2. When motionless food substances are the only ones present, the olfactory apparatus functions to a greater extent in eyeless larvae than it does in normal animals.

3. The experiments upon adult animals (table 1) indicate that, while the eye is the most important agent in obtaining food, yet with the nose alone the animals are capable of detecting and locating definite food substances.

4. When the animals are tested in darkness (table 1, series 3), the evident retardation in the time of reaction is due to the dependence of the animal upon the sense of sight.

5. After the removal of the eyes of the animals, they become accustomed to use the olfactory organ only and in this way the remaining sense organ is correlated to the needs of the animal.

6. Experiments performed with diffusing and non-diffusing substances (tables 2 and 3) demonstrate the fact that an animal possessing the optic sense is stimulated by non-diffusing substances, while those possessing the olfactory sense are stimulated by diffusing substances.

7. Odor streams involving the use of a number of substances (table 4) elicit no response on the part of the animal unless the substance used possesses irritating properties, in which case the animal responds by a decided motor reaction.

8. Responses to motionless test substances in air (table 1, series 3) show that the animal can find food substances, although the reaction time is longer than if the animal and the test substance are submerged.

9. The experiments indicate clearly that there is a definite olfactory sense in both the larvae and the adults of Amblystoma tigrinum.

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162 (1909)

Endomixis and encystment in Spathidium spathula.

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In a pedigree culture of *Spathidium spathula* there has been no indication of endomixis at any time during active vegetative life, and experiments were undertaken to determine the possibility of inducing an independent reorganization process in conjugating individuals by preventing nuclear interchange. Since conjugation in this race produces a significant increase in the division rate of the majority of exconjugant lines,[1] it was expected that a similar physiological effect would follow endomixis. Pairs in an early stage of fusion were separated by spurting from a pipette, and the division rates of lines derived from such split-conjugants were compared with those of normal exconjugant and non-conjugant lines.

Of seventeen exconjugant lines, thirteen produced an average of 7.3 generations more than the parent lines during the first fifteen days. Of thirty-nine split-conjugant lines, however, all but four produced essentially the same number of generations as the parent lines, indicating, apparently, that endomixis had not occurred. Additional evidence further supported this view.

Endomixis, however, has been observed in this race during encystment. Cytological study of encysted individuals at various stages in the process has revealed the fragmentation and resorption of macronuclear material, the persistence of micronuclei, and the formation of macronuclear anlagen. The physiological significance of endomixis during encystment has not been determined.

The complete paper will appear in the *Journal of Experimental Zoölogy*.

[1] L. L. Woodruff and Hope Spencer, Proc. Soc. Exp. Biol. and Med., 1921, xviii, 240.

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161 (1908)

Racial variations in Blepharisma undulans.

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Studies on *Blepharisma undulans* and *Blepharisma lateritia* Stein, have emphasized the impossibility or great difficulty of observing the micronuclei, and also the inevitable death of exconjugants without dividing. Thus Bütschli[1] was unable to identify micronuclei in vegetative specimens of *B. lateritia* though he found them in conjugants. Exconjugants invariably died. Calkins[2] did not find micronuclei in non-dividing *B. undulans*, but discovered them during division within the macronuclear membrane, and described their emergence and activities during conjugation. He likewise was unsuccessful in obtaining a single viable exconjugant.

During the past five months we have had under observation a pedigree culture of *Blepharisma undulans* which emphasizes racial differences within the species. The animals of this race possess from four to fourteen relatively conspicuous micronuclei, all of which are free in the cytoplasm during every stage of the life of the cell; and all the exconjugants thus far secured proved to be viable.

The main culture has attained one hundred and fifty generations to date, an average of about one division per day, in the standard beef extract culture medium used in this laboratory. At intervals, epidemics of conjugation have appeared. Exconjugants have been isolated and new lines established from their progeny. Studies are in progress similar to those which have been conducted on *Spathidium*.[3]

The wide experience of such observers as Bütschli and Calkins precludes the assumption that they were in error in regard to the

[1] Bütschli, *Abhandl. d. Senckenb. naturf. Gesellsch. Frankfurt a/M.*, 1876, Bd. x

[2] Calkins, *Journ. Morph.*, 1912, xxiii.

[3] Woodruff and Spencer, Proc. Soc. Exp. Biol. and Med., 1921, xviii, 180, 240

micronuclear condition of the animals which they studied and the only reasonable conclusion is that there are races of *Blepharisma* which differ in regard to the vegetative position of the micronuclei; in some it is intramacronuclear and others extramacronuclear—the present pedigree race of *Blepharisma undulans* representing the latter and therefore agreeing with the marine species, *Blepharisma clarissima*, as described by Anigstein. [1]

The results of all previous workers are consistent in regard to to the non-viability of exconjugants. Whether the intramacronuclear position of the micronuclei bears any causal relation to non-viability it is impossible to decide. Certainly there are no obvious differences between the chief nuclear phenomena described by Calkins in his non-viable conjugants and those which occur in the viable conjugants of the pedigree race which we are conducting.

[1] Anigstein, *Archiv. f. Protistenk.*, 1912, Bd. xxiv.

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1922, xix, p. 397.

195 (1942)

Rhythms in the rate of reproduction of Amœba bigemma.

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A specimen of *Amœba bigemma* (Schæffer) was isolated December 7, 1921, and eight lines of its descendants have been bred in pedigree cultures to the 100th generation (May 15, 1922). The cultures have been carried on standard beef extract[1] and kept at a temperature varying from 20° to 24° C. Vegetative division has been uninterrupted by spore or gamete formation. The data obtained by the daily isolation method show that the rate of division of the organism under the conditions of the experiment fluctuates considerably. At intervals during the pedigree there were daily divisions, or frequently one division every other day for a short time. Occasionally two fissions occurred in one day, while in a single instance an *Amœba* divided three times during twenty-four hours. Periods of inactivity when no fissions took place were common, and varied in length from one to twelve days; but whereas the absence of division for one or two days was of frequent occurrence, that of over three days was rare. An average of the daily divisions in eight lines for a period of ninety days showed the rate to be slightly above one division in two days.

In contrast to this daily irregularity there was a general periodicity in the fission rate which was revealed only by an examination of the data covering longer intervals of time. By averaging the number of divisions in a single line for five-day periods, a rhythmic character of the fission rate was apparent in the form of alternating periods of high and low reproductive activity; one low point to the next comprising about twenty days. Whether these rhythms are of the same nature as those in the Infusoria[1] can be determined only after more study of this and other *Amœbæ* in pedigree cultures.

[1] Woodruff and Baitsell, *Journ. Exper. Zoölogy*, 1911, Vol. 11.

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191 (1938)

Regeneration in Spathidium spathula and Blepharisma undulans.

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In a study of regeneration of form and function at various phases in the life history of the infusorians *Spathidium spathula* and *Blepharisma undulans*, it has been determined that the power of restoring lost parts and of continuing normal existence is in all cases dependent upon the same factor, the nuclear content of the fragment. Individuals of known pedigree were cut transversely, and the behavior or the fragments observed until regeneration and division or regeneration and death had occurred. During the vegetative state, regenerative power is highly developed in both ciliates, but because of the more distributed condition of the nucleus in *Spathidium* than in *Blepharisma* relatively smaller fragments from either extremity of the former are capable of complete regeneration. All fragments which continue to divide contain both macro- and micronuclear material. It has been impossible to produce an amicronucleate race by artificial means.[1]

In *Spathidium*, where the two nuclear elements extend throughout the cell both during and after fission, there is no difference in the regenerative power at any peroid of asexual life. In *Blepharisma*, however, where large amacronucleate fragments may be obtained during the process of division, it has been found that temporary restoration of external organelles is possible in the absence of nuclear material, if the formation of a new peristome has already been started. Dedifferentiation immediately follows and death occurs in a few days. The presence of micronuclei in such fragments, moreover, is not sufficient for continued existence.

During conjugation, complete regeneration in *Spathidium* is less frequent than during asexual life. Portions containing the synkaryon regenerate and divide; other pieces remain unchanged or undergo only temporary restoration of form. Fragments obtained from starved *Spathidia* prior to encystment either fail to regenerate, or regain normal form and subsequently encyst.

[1] Woodruff and Spencer, *Journ. Exper. Zoöl.*, 1922, Vol. 35.

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EXPERIMENTS ON THE DEVELOPMENT OF THE CRANIAL GANGLIA AND THE LATERAL LINE SENSE ORGANS IN AMBLYSTOMA PUNCTATUM[1]

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INTRODUCTION

The experiments set forth in this paper were undertaken for the purpose of extending our knowledge concerning the part which placodes and neural crest play in the formation of cranial ganglia and nerves, and also to determine, if possible, the extent of their

[1] Read before the American Association of Anatomists, March 25, 1921.

contribution to the formation of the mesoderm and the exact origin and fate of the 'mesectoderm' tissue. Experiments have never before been applied to the solution of these problems, all of our knowledge having been obtained from studies made upon successive developmental stages of normal embryos.

In the present study of the early stages of development it has been found that cells are given off for the formation of certain ganglia by epibranchial, lateral-line, gasserian, and ophthalmic placodes. From the early appearance of the neural crest in the dorsal portion of the neural canal at the time of the closure of the neural folds the crest cells have been followed as they migrate over the mesoderm of the visceral arches, increase in number and finally arrange themselves upon the median and ventral sides of this mesoderm, where they form the cartilages of the visceral skeleton except the second basibranchial. The distinctness of the early crest-cell groups and placodes makes it possible to remove these structures by employing the operative methods now so commonly used by investigators upon amphibian embryos. It is also possible to remove areas of ectoderm from early embryos in which the placodes have not yet become prominent enough to be identified, and thus to ascertain how early these placodes in the ectoderm are laid down as definite entities.

Much confusion arose in the earlier descriptions of placodes by the failure to distinguish clearly between ectodermal thickenings in relation to the gill clefts and those related to the formation of the lateral-line system. Not until von Kuppfer ('91) clearly pointed out the distinction between dorsolateral and ventrolateral, or epibranchial placodes, was there hope of obtaining light on the formation of the components of cranial ganglia. Descriptions of cells breaking off en masse from the epibranchial placodes and being added to portions of the cerebral ganglia have repeatedly appeared in the anatomical studies of all types of vertebrates, especially among the fishes and amphibians (van Wijhe, '82; Beard, '85; Froriep, '85; Platt, '96, and Landacre, '10 and '12). Landacre has assigned a definite function to these epibranchial placodes. He has shown in Ameiurus ('10) that the special visceral portion of the IX ganglion appears to come from

the epibranchial placode of the first true gill. Since Herrick ('07)[2] had found only gustatory fibers arising from the visceral ganglion of IX, Landacre naturally concludes that the epibranchial placode of IX gives rise to the ganglionic cells from which the gustatory fibers arise. He further expresses the opinion that this is the function of the epibranchial placodes in all types of vertebrates (Landacre, '12), since these placodes occur only on those nerves of VII, IX, and X which contain special visceral fibers, and since the relative size and growth of the epibranchial placodes in Lepidosteus and Ameiurus seem to be in relation to the area and time of appearance of taste buds supplied by fibers from special visceral ganglia. Coghill ('16) finds conditions in Amblystoma which seem to indicate that the visceral ganglia of VII, IX, and X all receive masses of cells from the epibranchial placodes.

There is also evidence that there are other placodes besides those concerned in the formation of lateral-line ganglia, i.e., general cutaneous placodes. Platt ('96) notes in Necturus that an ectodermal thickening above the eye appears to be concerned in some manner with the formation of the ophthalmicus profundus V nerve. A similar fact has been recorded by Coghill ('16) in Amblystoma and by Landacre ('12) in Lepidosteus, where in early stages of the formation of the ganglion of the ophthalmicus profundus V there is a distinct anchorage to the ectoderm over the eye. The former made the interesting observation that it was during this period, in which the anchorage is intact, that root fibers make their connection with the brain. However, neither of the latter investigators satisfied himself as to the significance of this early contact. Coghill ('16) also found in the earliest of his stages described, the 'non-motile,' an area of adhesion between the distal end of the gasserian ganglion and the skin. It is ventral to the primordium of the preauditory lateral-line organs and is not so extensive nor so intimate as the adhesion of the ophthalmic ganglion and skin.

[2] A note included in Landacre's paper ('07) by C. J. Herrick on the distribution of the IX nerve of Ameiurus melas. The conclusions from this reference are found in Landacre's paper ('12), page 3.

In connection with the general cutaneous component of X, Coghill ('16) finds in the 'non-motile' stage an intimate relation between an ectodermal thickening and a loose aggregation of cells upon the lateral aspect of the lateral-line ganglion of X. This cluster of cells, which he identifies as representing the jugular ganglion, is slightly more condensed in its most rostral portion where an incipient root is forming, which does not, however, at this stage reach the brain.

Lateral-line primordia. Platt ('96), Landacre ('10), Coghill ('16), Goette ('14), and others have shown that a different system of placodes is concerned in the formation of the lateral-line ganglia of VII, IX, and X. Not all of the investigators agree as to the exclusive derivation of these ganglia from placodes, for some have expressed the belief that neural crest also enters into their formation.

The primordia of the lateral-line system have been shown very clearly by Platt ('96), Landacre ('10), and Landacre and Conger ('13) to have origins independent of the auditory vesicle. However, anterior and posterior prolongations of the auditory thickening have been observed in many forms, and to these Landacre ('10) applies the term of 'preauditory and postauditory' placodes. He does not have a clear idea as to the relation of these placodes to the sensory lines, for in the case of the preauditory placode (Landacre and Conger, '13) it seemed to disappear by a process of degeneration before the appearance of the lateral-line primordia, and in the case of the postauditory placode ('10) it loses the characteristic cell arrangement and does not give rise to the lateral-line organs. Moreover, in Lepidosteus Landacre and Conger ('13) fail to find any postauditory placode.

Although separate origins have been given to each of the groups of the lateral-line sense organs by various investigators, none have described such an early pattern of the lateral-line system as the one observed in Necturus. According to Platt ('96, p. 491), the plan of this system is early laid down in three longitudinal lines on each side of the embryo, connected by intersegmental cross-lines with special differentiations at points of intersection, and out of this pattern certain portions are retained in the

final system. However, when one compares this description with skin amounts of early stages of Amblystoma, another interpretation seems to be more plausible. No such pattern can be found in Amblystoma embryos, but whenever the contour of underlying structures makes its appearance on the surface certain transitory thickenings appear due to mechanical molding, as one finds between somites, gill swellings, around the early optic vesicles and the early limb bud. It seems possible, therefore, that most of the early pattern described in Necturus has no significance in the formation of the lateral-line system.

Neural crest. It is generally accepted that portions of the cranial ganglia are derived from the neural crest; in fact, early investigators of cranial nerve problems made the crest cells their sole source of origin. Landacre ('10) would derive all general cutaneous ganglia and the general visceral system exclusively from neural crest.

In view of the fact that there have appeared descriptions of extensive wanderings of neural crest, it seems strange that so little emphasis has been put upon such an outstanding feature. The literature contains comparatively few descriptions of any complete investigations of the growth of the neural crest outside of its supposed connection with the formation of cranial ganglia. In most of the descriptions of the early stages of developing cranial ganglia the assumption that the crest cells were concerned only with the formation of ganglia and nerves has been so general that many investigators have been led to overlook the fact that any further wandering of these elements occurs. However, various careers have been assigned to them by a few investigators.

Marshall ('78), working on the chick, and van Wijhe ('82), on the selachians, were among the first to observe that the anterior part of the neural crest, which could not be identified with the formation of ganglia, gradually disappeared. They could not determine its final fate. Kastschenko ('88) went a step farther in suggesting that the cells which were lost from the neural crest did not degenerate, but added themselves to mesenchyme. Additional descriptions of the neural crest then followed, by Gorono-witsch ('93) in birds and Platt ('96, '97) in Necturus. Both

agreed that a greater part of the neural crest, augmented at the margin of the gill clefts by a proliferation from the lateral ectoderm, becomes a wandering mass of ectodermal cells to which Platt gave the name 'mesectoderm' and that this wandering mass of ectodermal cells is the origin of certain mesenchymal tissue. It was Platt, however, who suggested that the branchial cartilages, surrounding connective tissue, and the anterior portions of the trabeculae were derived from the 'mesectoderm.' In respect to the branchial cartilages, this agrees with von Kuppfer ('95) on Petromyzon, although he claims that the wandering ectodermal cells are derived from deeper layers of ectoderm in situ. Furthermore, Dohrn ('02), working on Torpedo, and Brauer ('04), on the Gymnophiona, agree in the main with Platt's description of the mode of formation of branchial cartilages, but they ascribe the sole origin of the cartilages to the neural crest.

Recently Goette ('14) has discussed the formation and development of the 'ectomesoderm,' as he calls it, in Siredon (Amblystoma tigrinum) which also illustrates what he finds in Petromyzon and Torpedo. He confines the anlagen of the 'ectomesoderm' to the ectoderm in the regions of V, VII, IX, and X, which he claims forms visceral skeleton, the outer gill muscles, and the surrounding connective tissue. To the neural crest, epibranchial placodes, and lateral-line placodes belongs the function of the formation of the cranial ganglia and nerves.

From this summary of the literature it is evident that there is much disagreement concerning the formation of cranial ganglia and the origin and further distribution of the wandering masses of cells of ectodermal origin.

In the light of these facts, an investigation of this problem was suggested by Prof. R. G. Harrison on an animal, such as Amblystoma punctatum, which would lend itself to an experimental analysis of all the factors involved. It gives me pleasure to express here my appreciation to Doctor Harrison for his kind criticisms and suggestions during its progress.

Since the completion of the present investigation, there has appeared a paper by Landacre ('21) on the fate of the neural crest in Plethodon glutinosus, a urodele. In so far as the visceral

cartilages are concerned, the morphological findings in this paper correspond very closely to the observations recorded in the present study of Amblystoma. In Plethodon the manner of the migration and disposition of the neural crest upon the branchial arches is similar to that described by Platt ('97). In addition to contributing to the ganglionic portions of V, VII, IX, and X, the neural crest is described as forming the greater portion of the mesenchyme in the ventral part of the head and in the branchial region, the anterior portions of the trabeculae, Meckel's cartilage, the palatoquadrate bar, and all the branchial cartilages except the second basibranchial.

MATERIAL, METHODS, AND NORMAL DEVELOPMENT

All the experiments have been made upon embryos of Amblystoma punctatum in stages ranging from 21 to 27 as shown in figures 1 to 4. The technique employed in the operation is similar to that already described by Harrison ('18). The special types of operation employed in this investigation will be subsequently described under separate sections.

In order that the experiments may be more clearly interpreted, a description of the stages in the normal development of the neural crest and of certain placodes will first be given. This description was obtained chiefly from a series of dissections supported in the more minute details by serial sections. In order to accomplish the dissections, embryos which had been preserved in the ordinary corrosive sublimate acetic mixture were placed in a 5 per cent aqueous solution of nitric acid, where they were kept from twelve to twenty-four hours for the purpose of softening the brittle ectoderm and at the same time rendering it pliable. They were then placed under the binocular microscope, and by means of a small pair of operating scissors and suitable needles a continuous incision was made up the middorsal line and down the midventral line. The two halves of ectoderm were carefully removed, stained lightly with haematoxylin, cleared in the usual manner, and mounted in damar on glass slides. After such treatment the positions of ectodermal thickenings can be observed accurately.

The dissected embryos were in some cases preserved in glycerin after they had been stained with haematoxylin. However, the neural crest may be more satisfactorily observed unstained in water or alcohol, because its grayish-brown color sufficiently differentiates it from the surrounding tissue up to a certain stage as will be described later.

Although the picture of the crest cells and placodes is approximately constant for each stage, occasionally normal embryos whose external appearances indicate similar age disclose upon dissection noticeable differences in the early rate of growth of the crest cells or placodes. This difference may even be confined to the same individual where, for example, the advance of the trunk lateral-line primordia of the one side lags conspicuously behind the primordia of the other side for a distance of one or two somites.

Stage 21

Neural crest. A layer of neural crest, light brown in color, over the dorsal and dorsolateral portions of the medullary tube extends from a point above the eye to a point above the posterior border of the second somite (fig. 1) where it is continuous with the crest cells in the spinal region. Ventrally there are two distinct proliferations separated by a constriction lying approximately midway between the two ends. The more anterior proliferation becomes the band which later wraps around the mesoderm of the hyoid arch.

Where the dorsal margin of the mesoderm of the branchial region meets the lateral surface of the medullary tube there is a trough-like depression, filled in by a deep longitudinal ridge of ectoderm extending from above the primitive hyomandibular cleft to near the first somite, where it becomes continuous with less marked ridges over the somites. This is represented by dotted lines in the figure.

Figure 13 is a frontal section passing through the longitudinal ectodermal ridge and the anterior and posterior extremities of the layer of neural crest. The considerable thickness of the ectodermal ridge can be seen on either side in the region of the

hind-brain. A cap of crest cells, whose base extends as a wedge between the fused neural folds, lies in the region of the mid-brain. These cells are represented by well-formed nuclei surrounded by a pigmented cytoplasm in which lie fine, closely packed yolk granules which are similar in size to those of the neural canal. The mass of cells lies close to the ectoderm and extends laterally a short distance over the walls of the mid-brain. Over the region of the hind-brain a similar small cap can be seen lying in the same relation to the ectoderm and brain, except that its base does not appear in this plane of section to be wedged between the fused folds.

Placodes. Figure 1 shows an area over the dorsal border of the eye, which represents the position of a very slight thickening of the ectoderm where cells are given off later to the ophthalmic ganglion. It may be said here that throughout the different stages studied, this ectodermal thickening never acquires a thickness comparable to that of other placodes. Many times in the dissections the area was thinner than the surrounding ectoderm because of the fact that its cells adhered to the loose ganglionic mass of cells forming the ophthalmic ganglion. This thickening may be called the ophthalmic placode.

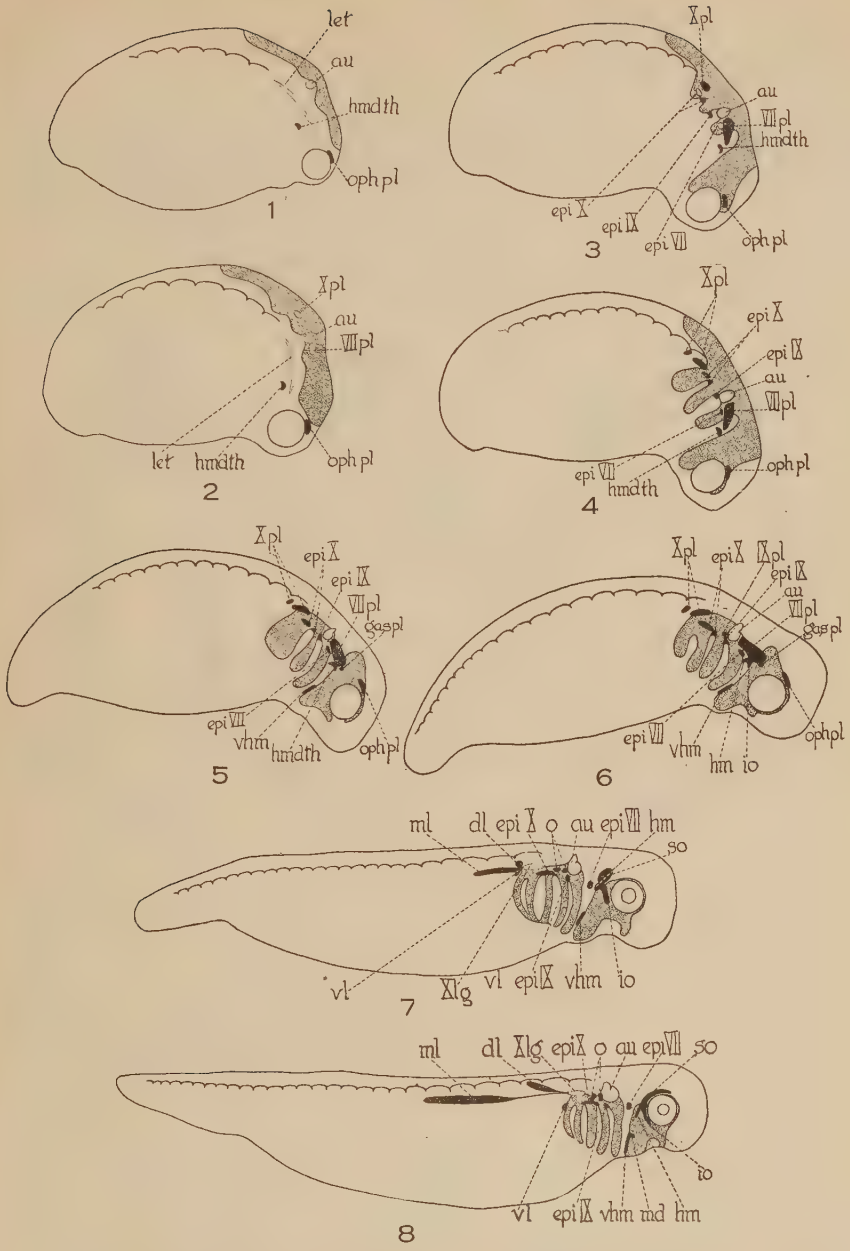
Below the anterior extremity of the longitudinal ridge lies a small ectodermal thickening in the dorsal extremity of the hyomandibular cleft, which can be detected at this stage. This small ectodermal thickening takes on the contour of the cleft at this point, giving it approximately a crescent shape. In the position of the anterior portion of this thickening there later arises the primordium of the infra-orbital group of lateral-line sense organs, while in the position of the posterior portion there later arises the hyomandibular group.

Stage 23

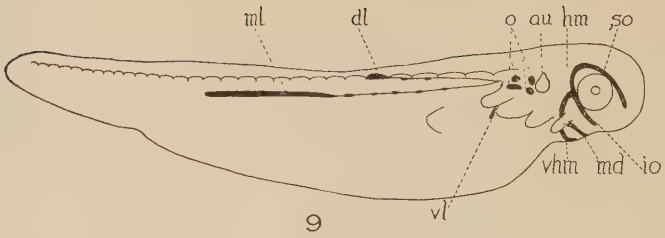
Neural crest. This stage shows a decided ventral growth of the neural crest over the lateral walls of the brain (fig. 2). Above and posterior to the dorsal border of the eye an extensive proliferation has taken place, carrying the neural crest downward toward the primitive mandibular arch. Figure 14 shows above

ABBREVIATIONS

alv., alveolaris VII nerve
an., angular group of sense organs
au., ear
buc., ramus buccalis VII
br., brain
bv., blood vessel
c. c., neural-crest cells
ch., notochord
chy., ceratohyal
dl., dorsal body line of sense organs
e., eye
ect., ectoderm
epi. VII., epibranchial placode of VII
epi. IX., epibranchial placode of IX
epi. X., epibranchial placode of X
ex. g., external gill
gas. g., gasserian ganglion
gas. pl., gasserian placode
jg., jugularis VII nerve
hh., hyohyal
hm., hyomandibular group of sense organs
hmd. th., ectodermal thickening in dorsal hyomandibular cleft
hy. a., mesoderm of hyoid arch
hy. cc., hyoid crest-cell group
hy. m., hyoid muscles
io., infra-orbital group of sense organs
l. e. t., longitudinal ectodermal thickening
mb., mandible
md., mandibular group of sense organs
md. a., mesoderm of the mandibular arch
md. cc., mandibular group of crest cells
md. m., mandibular muscle
md. V., truncus mandibularis V
ment., mentalis VII nerve
m. l., midbody line of sense organs
ms., mesoderm
na., nose
o., occipital group of sense organs
op. v., optic vesicle
oph. g., ophthalmic ganglion
oph. pl., ophthalmic placode
oph. p. V., ophthalmicus profundus V nerve
ph., pharynx
pl., palatinus VII nerve
q., quadrate
s., somite
so., supra-orbital group of sense organs
sup. VII, ramus ophthalmicus superficialis VII
th. m., thoracicohyoideus muscle
tmp. m., temporalis muscle
tr., trabecula
v. hm., ventral hyomandibular group of sense organs
vl., ventral body line of sense organs
1–2 bb., first and second basibranchial
1 bb. cc., first basibranchial crest-cell group
1–4 br. a., mesoderm of first to fourth branchial arches
1–4 br. cc., first to fourth branchial crest-cell groups
1–2 cbr., first and second ceratobranchial
1–4 ebr., first to fourth epibranchial
1 s., first somite
1 int. r., first intersomitic ridge
VII g., facial ganglion
VII l. g., facial lateral-line ganglion
VII pl., facial lateral-line placode
IX lg., glossopharyngeus lateral-line ganglion
IX pl., glossopharyngeus lateral-line placode
IX vis. g., glossopharyngeus visceral ganglion
X lg., vagus lateral-line ganglion
X pl., vagus lateral-line placode
X vis. g., vagus visceral ganglion
X vis. t., vagus visceral trunk



Figs. 1 to 8 Camera-lucida drawings of stages 21, 23, 25, 26–27, 30, 33, 35, and 35+ of embryos of Amblystoma punctatum, showing development of neural crest, ectodermal placodes, and primordia of lateral-line sense organs. Neural crest is stippled and placodes and primordia are solid black. × 10. For abbreviations see page 430.



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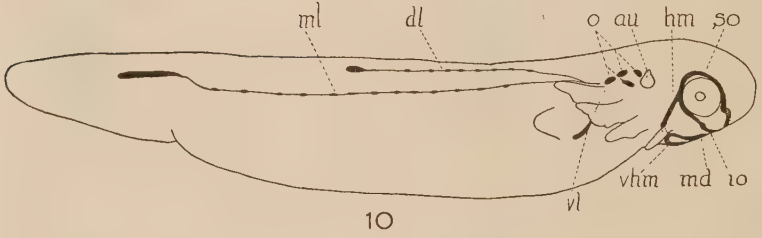

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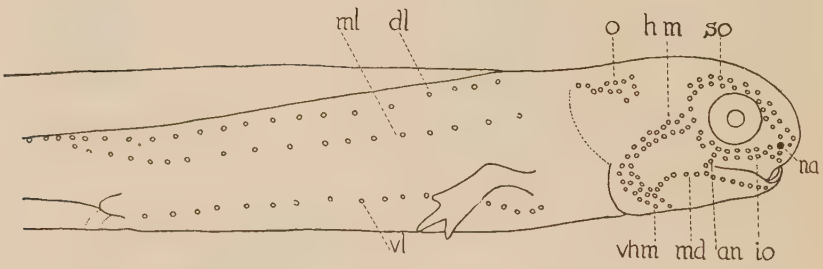

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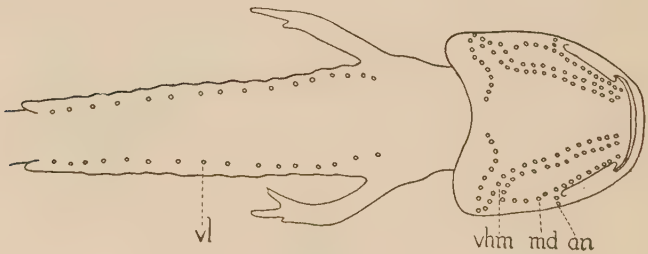

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Figs. 9 and 10 Camera drawings of stages 36 and 37–38, showing further development of lateral-line primordia. × 10.

Figs. 11 and 12 Camera drawings of lateral and ventral views, respectively, of stage 46+, showing final distribution of lateral-line groups of sense organs. The gills have been removed. × 10. For abbreviations see page 430.

the longitudinal ectodermal ridge small portions of the neural crest descending upon the lateral walls of the hind-brain connected dorsally by a cap of cells still wedged tightly between the ectoderm and brain.

Posterior to the mandibular arch are the two prolongations of the neural crest which appeared in stage 21. Approaching the

Fig. 13 Frontal section passing through longitudinal ectodermal ridge and anterior and posterior extremities of layer of neural crest in stage 21, showing origin of crest cells from dorsal and median portions of neural folds. × 37.

Fig. 14 Cross-section passing through longitudinal ectodermal ridge and ectodermal thickening in dorsal portion of hyomandibular cleft in stage 23, showing neural crest over dorsal portion of neural tube and portions of neural crest descending to lateral walls of neural tube. × 37.

Fig. 15 Cross-section through upper half of optic vesicles in stage 25, showing crest cells descending along sides of neural tube and over mesoderm of mandibular arches and over anterior portion of right optic vesicle. × 37.

first somite is an additional proliferation which later covers the mesoderm of the second, third, and fourth branchial arches. In this stage the neural crest has extended posteriorly (fig. 2) to a position above the anterior half of the fourth somite.

Placodes. The ophthalmic placode lies in relatively the same position as in stage 21. Its indefinite borders outline a slightly

thickened portion of the ectoderm which is in close contact with the crest cells at its posterior border. The longitudinal ectodermal ridge (fig. 2) is still prominent, but extends slightly more anteriorly and less posteriorly than in the previous stage. The thickening in the dorsal portion of the hyomandibular cleft is slightly larger, but still remains crescentic in shape (fig. 14). The approximate positions of two placodes, anterior and posterior to the probable position of the auditory placode, are indicated by dotted lines. That which lies just anterior to the auditory placode gives rise to part of the lateral-line ganglion of VII. That which lies posterior to the auditory placode gives rise to part of the lateral-line ganglion of X. The positions of other placodes cannot be exactly located in sections of this stage by the appearance of any ectodermal thickenings.

Stage 25

Neural crest. A further very rapid ventral growth of the neural crest has taken place, the most pronounced proliferation involving those cells which are in the region over the dorsal border of the eye (fig. 3). The upper portion of the mandibular arch as far as the middle of the posterior border of the eye is covered by the neural crest. A growth of the neural crest passes over the dorsal border of the eye. A section passing through the upper half of the optic vesicles (fig. 15) shows on one side along the anterior border of the latter a transection of the ventral proliferation of the crest cells lying close to the ectoderm. Posterior to the optic vesicles on either side may be seen the descending crest cells loosely packed together, wrapping around the anterior and lateral surfaces of the mesoderm of the mandibular arch. Over the dorsal border of the hind-brain region lies a thin layer of crest cells which is continued upon the lateral walls as a more compact band of cells. This group of crest cells descends along the anterior border of the auditory placode to the mesoderm of the hyoid arch. A distinct though more slender band of crest cells along the posterior border of the auditory placode approaches the mesoderm of the first branchial arch, extending as far ventrally as the anterior band. Still further

posteriorly, a somewhat broader proliferation, covering the anterior border of the first somite, descends ventrally on the gill mesoderm to the level of the crest cells on the first branchial arch. The neural crest continues posteriorly above the dorsal border of the somites along the dorsolateral border of the neural canal to a position in the middorsal line above the middle of the third somite.

Placodes. The longitudinal ectodermal ridge has disappeared at this stage, due to the rapidly descending neural crest and the growth of the visceral arches. The extent of the area of the ophthalmic placode is about the same as in stage 23. The thickening in the dorsal portion of the hyomandibular cleft has changed but little in size. A placode approximately triangular in shape, situated anterior to the auditory placode, can be seen rapidly approaching the hyomandibular cleft from above. This is the placode which gives rise to a large part of the lateral-line ganglion of VII by contributing large numbers of placodal cells from its posterior extremity (fig. 16). The epibranchial placode of VII, judging from its position in subsequent stages, is probably situated just below the posterior border of this placode. This probable position is circumscribed by dotted line in figure 3. On the posterodorsal border of the hyomandibular cleft is a slight thickening of the ectoderm, which is identified as the epibranchial placode of IX. However, no placodal cells are splitting off from the ectodermal thickening to form a ganglion (fig. 16). In the dorsal portion of the first branchial cleft there is a small thickening of the ectoderm, which is possibly the position of an epibranchial placode, which gives rise to part of the sensory visceral ganglion of X, although no cells are splitting off at this stage. The possible position of the other epibranchial placode of X is also circumscribed by dotted line in the figure. Posterior to the auditory placode and anterior to the first somite, a slight thickening in the ectoderm is identified as the placode which gives rise to most of the vagus lateral-line ganglion.

Stage 26–27

Neural crest. In the description of the following stages, the descending bands of neural crest will be designated by the visceral arches upon which they lie.

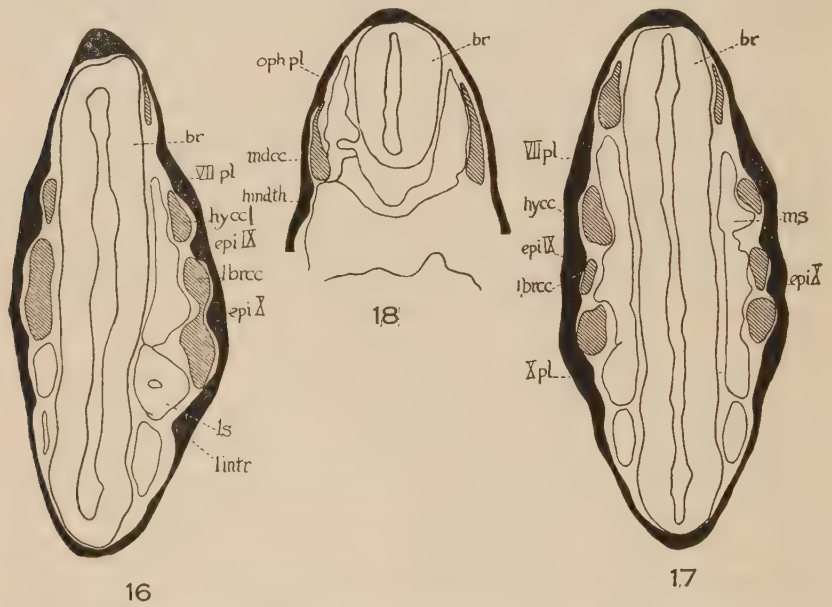


Fig. 16 Frontal section, showing in stage 25 crest cells over dorsal portion of right optic vesicle and over mesoderm of hyoid and branchial arches. Also shows positions of VII lateral-line placode and epibranchial placodes of IX and X. × 37.

Fig. 17 Frontal section, showing crest cells migrating over mesoderm of hyoid and branchial arches and above optic vesicles in stage 26–27. Also shows positions and relative thickness of VII and X lateral-line placodes and epibranchial placodes of IX and X. × 37.

Fig. 18 Frontal section, showing ophthalmic placode on right side lying close to anterior portion of neural crest migrating upon mesoderm of mandibular arch in stage 26–27. × 37.

Further rapid growth of neural crest has taken place ventrally, accompanied by a thinning out of the crest cells over the middorsal portion of the brain (fig. 4). The anterior border of the eye is traversed as far as the dorsal border of the nasal placode by a narrow strip of loosely arranged cells. The mesoderm of the

mandibular arch is covered by crest cells to a level below the ventral border of the eye. Sections show that the crest cells are beginning to wrap around the mesoderm of the arch—a condition which can be seen more clearly in stage 30. The hyoid and first branchial crest-cell groups have extended farther ventrally to about the same level. The hyoid group (fig. 17) is quite thick and packed tightly against the arch. The first branchial group is more slender and curves slightly toward the hyoid arch. The remaining portion of the branchial mesoderm is being covered by a band of the neural crest which is slightly constricted at its union with the neural crest above. The posterior extension of the neural crest is diminished as its cells migrate toward the gill region, the extremity reaching only to a point above the posterior border of the second somite. It is from this posterior portion above the first three somites that the crest cells of the second, third, and fourth branchial arches have their source.

Placodes. Over the dorsal border of the eye the ophthalmic placode is a long narrow thickening which gives the appearance of having a broad contact with the loose neural crest against which it lies (fig. 18). The triangular placode anterior to the auditory placode approaches more closely the dorsal border of the crescent-shaped thickening above the hyomandibular cleft (fig. 4). The posterior margin of the placode of the lateral-line ganglion of VII is giving off a large number of cells which approach the neural crest lying along the anterior border of the auditory placode. The epibranchial placode of VII is situated immediately below and anterior to this cell proliferation as a very slight ectodermal thickening lying close to the lower border of the lateral-line placode. The epibranchial placode of IX lies in a level somewhat dorsal to its position in stage 25. On its inner surface loose cells containing mitotic figures lie closely against the crest cells descending on the anterodorsal margin of the first branchial arch, indicating that placodal cells are being given off to form a ganglion (fig. 17). Posterior to the placode which lies in the upper extremity of the first branchial cleft a somewhat elongated area of the ectoderm is slightly thickened. It is very probable, judging from the study of older stages, that these placodes are the

epibranchial placodes which contribute placodal cells to form the visceral sensory ganglion of X. The placode of the vagus lateral-line ganglion and of the midbody line is situated posterior to its former position and lies across the anterior border of the first somite above the neural crest of the second, third, and fourth branchial arches (fig. 17). In the ectodermal intersegment of the first and second somites is a prominent thickening, which later is the position of the placode which gives rise to the dorsal body line of sense organs and a portion of the vagus lateral-line ganglion.

Stage 30

Neural crest. This stage shows the rapid descent the neural crest has made in its progression ventrally. No longer are the crest cells from both sides continuous over the dorsal border of the brain (fig. 5). The crest cells over the mesoderm of the mandibular arch and over the dorsal and anterior borders of the eye have broken all connection with those of the hyoid arch. The maxillary and mandibular processes of the mandibular arch have become covered with crest cells. The manner in which the mesoderm of the visceral arches is becoming completely surrounded by a layer of crest cells two to three cells thick is shown in figure 19. The mesoderm of the mandibular and hyoid arches is completely surrounded by the crest cells, while the first branchial arch is enclosed around its anterolateral and posterior borders. The crest cells over the anterior border of the eye now extend as a narrow band to the dorsal border of the nasal placode. A few scattered crest cells are found along the dorsal border of the auditory vesicle which are continuous with crest cells lying on its anterior and posterior borders. A few crest cells come to lie below the ventral border of the auditory vesicle, due to the rapid ventral shifting of crest cells from above. The crest cells from the anterior border of the auditory vesicle continue with those of the hyoid arch, which have descended ventrally farther than the crest cells of the first branchial group. The dorsal region of the crest cells lying posterior to the auditory vesicle consists of loosely arranged cells which extend to the middle of

the first somite. All of the crest cells are then confined to the broad band which now descends over the mesoderm of the second, third, and fourth branchial arches.

Placodes. The ophthalmic placode is still an elongated thickening (fig. 20), in which may be seen mitotic figures. Its posterior border is in close contact with the neural crest. It gives

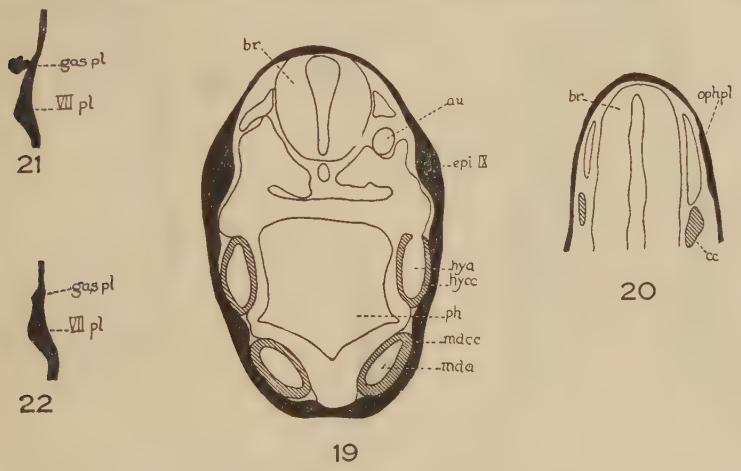


Fig. 19 Cross-section, showing in stage 30 the manner in which crest cells are completely surrounding mesoderm of hyoid and mandibular arches. Also shows ectodermal thickening in dorsal portion of hyobranchial cleft containing epibranchial placode of IX. × 37.

Fig. 20 Frontal section of elongated ophthalmic placode in stage 30. × 37.

Fig. 21 Frontal section of stage 30, showing point of contact of gasserian ganglion near its distal end with a small ectodermal thickening at anterior extremity of VII lateral-line placode. × 37.

Fig. 22 Frontal section, showing in stage 28 a small ectodermal thickening (gasserian placode) slightly below the anterior extremity of VII lateral-line placode. × 37.

the appearance of contributing placodal cells to the ophthalmic ganglion.

Near the anterior extremity of the elongated placode of VII there is a slight thickening in the ectoderm. A few loose cells are connected with the inner side of the thickening (fig. 21). This area may be a small placode concerned with the gasserian portion of V. The only similar area in stages earlier than this

can be found in stage 28. There is a slight thickening in the ectoderm in front of and slightly below the anterior extremity of the VII placode (fig. 22). The placode of the lateral-line ganglion of VII has extended to the dorsal border of this placode, with which it fuses near the dorsal portion of the hyomandibular cleft.

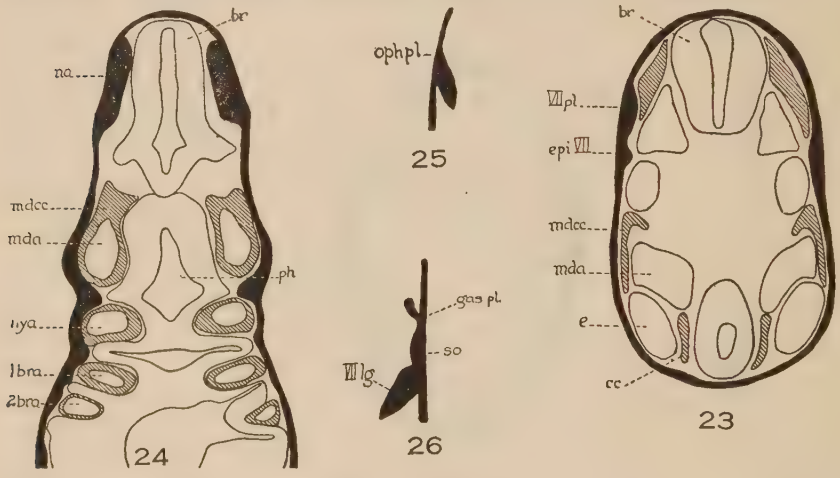


Fig. 23 Cross-section, showing in stage 30 the epibranchial placode of VII lying below lateral-line placode of VII and also crest cells descending over mandibular arch and dorsal portion of optic vesicles. × 37.

Fig. 24 Frontal section through lower portions of mesoderm of mandibular, hyoid, first and second branchial arches in stage 33, showing manner in which arches are being completely surrounded by crest cells. × 37.

Fig. 25 Frontal section, showing in stage 33 broad contact of ophthalmic ganglion with ectoderm. × 37.

Fig. 26 Frontal section, showing in stage 33 contact of distal portion of gasserian ganglion with an ectodermal thickening at anteroventral margin of supra-orbital primordium. × 37.

The posterior extremity of the VII lateral-line placode is contributing cells to a mass lying close upon the anterior border of the auditory vesicle. Many mitotic figures can be seen in this portion where the cells are separating from the placode. Above the point of fusion of the placode of VII with the placode near the dorsal portion of the hyomandibular cleft, the ectodermal thickening becomes broadened in the anterodorsal direction

(fig. 5). In the lower portion of the hyomandibular cleft, on a level with the ventral border of the optic vesicle, appears an elongated ectodermal thickening, where later primordia of the ventral hyomandibular and mandibular groups of lateral-line sense organs appear.

The epibranchial placode of VII can be seen as a small ectodermal thickening somewhat deeper than in stage 26–27, lying on the upper anterior border of the hyoid arch just below the lateral-line ganglion placode of VII (fig. 23). The epibranchial placode of IX appears in approximately the same position as in the previous stage and is preparing to give off a mass of cells (fig. 19). The vagus lateral-line placode has begun to give off a large mass of cells which comes in contact with a thin layer of the neural crest. Above the dorsal extremity of the first branchial cleft is the small epibranchial placode which is a visceral sensory placode of the vagus. In the region below the lateral-line ganglion placode of the vagus the ectoderm is somewhat thickened and represents the posterior portion of the visceral sensory placode of the vagus. In the intersegment of the first and second somites, the prominent ectodermal thickening appears somewhat larger than in stage 26–27.

Stage 33

Neural crest. The rapidly diminishing area of neural crest in the region over the dorsal portion of the eye is seen in this stage (fig. 6). The crest cells have passed farther behind the nasal placode on its posterior border where they join, along the ventral border of the optic vesicle, those which surround the maxillary process. The crest cells over the dorsal border of the hyoid arch are continuous with a broad area of crest cells which lie upon the anterior and ventral borders of the auditory vesicle. The mesoderm of the hyoid and first branchial arches is covered by crest cells which have now extended to the ventral borders of these arches. The crest cells lying above the remainder of the branchial region have diminished in number and now lie in a thin layer. The neural crest can no longer be found over the dorsal border of the auditory vesicle. The broad sheet of crest

cells which appeared in stage 30 over the posterior two-thirds of the branchial region has now split into two bands, the anterior of which is covering the second branchial arch, while the posterior shorter band lies on the mesoderm of the third and fourth branchial arches.

In the lower portion of the visceral arches (fig. 24) the mesoderm of the mandibular, the hyoid, and the first and second branchial arches can be seen on either side entirely surrounded by neural crest. The third branchial arch which appears in more dorsal sections is likewise entirely surrounded by neural crest. As the sections are followed ventrally, many mitotic figures can be seen in these rings of neural-crest cells, indicating a rapid increase in the number of cells as well as rapid migration. They have grown very abundant in their position around the visceral arches, especially so around the anterior portion of the mandibular arch where they are continuous ventrally in front of and behind the stomadaeum upon the maxillary and mandibular processes.

Placodes. The ophthalmic ganglion, at its anterior extremity, has a wide contact with the ectoderm (fig. 25). Mitotic figures appear on the ectodermal side of the contact throughout this and other sections. The extent of the contact is indicated in figure 6. The gasserian ganglion near its distal end has a definite contact, for about four sections, with the ectoderm at the anteroventral border of the VII lateral-line placode (fig. 26). This is possibly a placode for the gasserian ganglion. The actual contact is very brief and its history is difficult to follow. In later stages it is not possible to detect the placode. Many placodal cells forming the lateral-line ganglion of VII are splitting off from the posterior portion of the elongated placode which lies above the hyoid arch and hyomandibular cleft (fig. 6). The lower portion of the lateral-line ganglion, which is thus forming, consists of cells less compact at this stage. These later supply the hyomandibular division of the lateral-line component of VII. Where the VII lateral-line placode fuses with the placode in the dorsal portion of the hyomandibular cleft (fig. 6) the anterodorsal extension is larger than in stage 30. In the epibranchial placode of VII are

numerous mitotic figures showing a rapid development of placodal cells (fig. 27). It is difficult to mark off the upper portion of this placode because of its proximity to the ventral border of the loose cells of the lateral-line ganglion above. The primordium of the

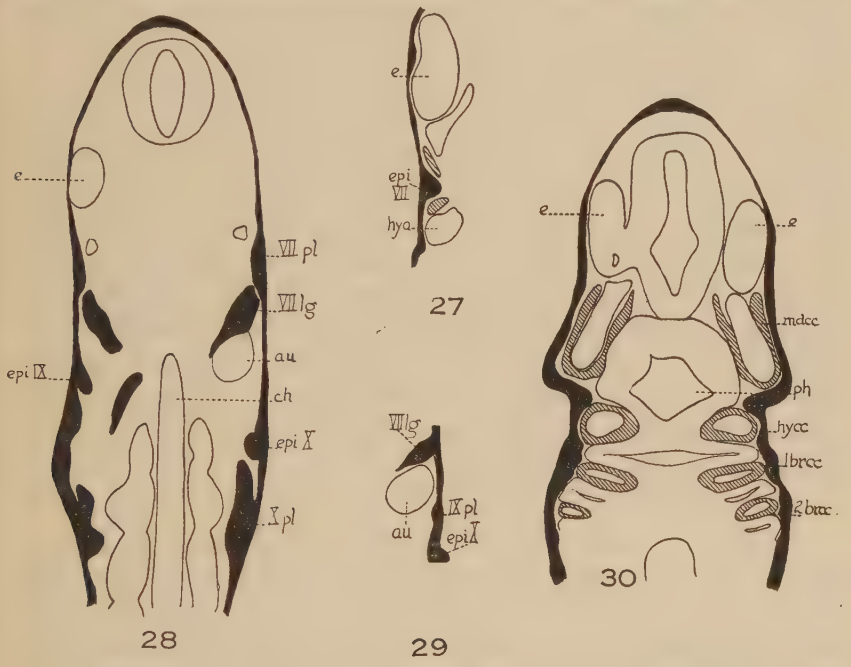


Fig. 27 Frontal section, showing epibranchial placode of VII in stage 33 proliferating a large mass of cells. × 37.

Fig. 28 Frontal section, showing in stage 33 lateral-line ganglion and placode of VII, rounded epibranchial placode of IX on left side and epibranchial placode of X on right anterior to X lateral-line placode in process of forming ganglion. × 37.

Fig. 29 Frontal section, showing at stage 33 lateral-line placode of IX posterior to auditory capsule. × 37.

Fig. 30 Frontal section, showing at stage 35 crest cells surrounding mesoderm of mandibular, hyoid, first and second branchial arches and also increase in number of crest cells on median side of hyoid arch. × 37.

ventral hyomandibular line of sense organs in the lower portion of the hyomandibular cleft is somewhat elongated and deeper than in stage 30. The epibranchial placode of IX (fig. 28) is giving off a rounded mass of cells just above and slightly posterior

to the hyobranchial cleft. Above the epibranchial placode of IX there appears a small round thickening, possibly the IX lateral-line placode, in the ectoderm near the posteroventral border of the ear. It merges into thickened ectoderm which posteriorly is identified as the epibranchial placodal region of X (fig. 29). Above the first branchial cleft a placode is also giving off cells in large numbers which lie close to the neural crest. It is connected by loose cells which lie along the ventral border of the vagus lateral-line ganglion next to a thickened portion of the ectoderm (fig. 6). This entire mass of cells, including the placode in the dorsal portion of the first branchial cleft, is the placodal mass which gives rise to the visceral sensory ganglion of X. The anterior extremity of the elongated placode over the posterodorsal border of the crest cells of the branchial region is splitting off a large mass of placodal cells to form the vagus lateral-line ganglion (fig. 28). The placode in the intersegment of the first and second somites has increased somewhat in size.

Stage 35

Neural crest. The remains of neural crest over the dorsal portion of the eye exist only as a very narrow band of loose cells which are continuous anteriorly as far as the nasal placode, and posteriorly as far as the mandibular crest cells with which they are united (fig. 7). The posterior border of the nasal placode is covered by a group of crest cells continuous with the maxillary group, which in turn joins ventrally in front of the stomadaeum with the maxillary group of the opposite side. Only a few loose crest cells are still visible along the anterior border of the auditory vesicle. The crest cells extend to the ventral extremity of the mesoderm of the hyoid arch near the pericardium. Above the gill region and posterior to the auditory vesicle, a narrow longitudinal band of crest cells extends to near the posterior border of the first somite. Descending ventrally are bands of crest cells which supply each of the branchial arches. The recent splitting of the broad most posterior band of crest cells, which existed in stage 30 (fig. 6), is indicated by the ventral continuations of the crest-cell bands of the third and fourth branchial arches.

A few loose crest cells appear to join the ventral extremities of the second, third, and fourth branchial arches.

A new feature in the rapidly migrating crest cells now appears. They begin to accumulate along the median side of the arches where many mitotic figures can be seen, especially in the lower halves of the latter. The typical condition is seen in the section on the median side of the hyoid arch. At this level the mandibular arch is not completely surrounded by crest cells on its anterior border as it is farther ventrally. If the sections are studied still farther ventrally, it can be seen that the crest cells are continuous across the ventral extremities of the mesoderm of the hyoid arches in front of the pericardium and across the ventral extremities of the mandibular and maxillary processes behind and in front of the stomadaeum. If the sections are studied farther dorsally, all the branchial arches show, along their ventral halves, the condition similar to that of the hyoid arch (fig. 30).

Placodes. No longer is there any contact of the ophthalmic ganglion with the ectoderm, although its anterior end lies close to it. All traces of an ectodermal thickening in this region have disappeared, leaving the ectoderm the same thickness as before its appearance.

The primordium of the supra-orbital line of sense organs now appears as a prominent ovoid placode whose anterior end is directed anterodorsally (fig. 7). Its posterior border is joined by the incipient ramus ophthalmicus superficialis VII, while its ventral border is connected with the ectodermal thickening in the dorsal portion of the hyomandibular cleft. Along the course of the anterior extremity of the ectodermal thickening, which was shown in stage 33, there now descends near the posterior border of the optic vesicle a well-defined primordium of the infra-orbital line of sense organs. Along the posterior extremity of the ectodermal thickening is the small primordium of the hyomandibular group of sense organs which is connected to the incipient ramus buccalis VII.

The epibranchial placode of VII lies just posterior to the primordium of the hyomandibular group of sense organs and upon the posterior portion of the hyomandibular cleft (fig. 7). It has

a broad contact with the distal end of the visceral sensory portion of VII extending dorsoventrally in the cleft. Many mitotic figures may be found at or near the point of contact, indicating that many placodal cells are still steadily being split off to form the ganglion.

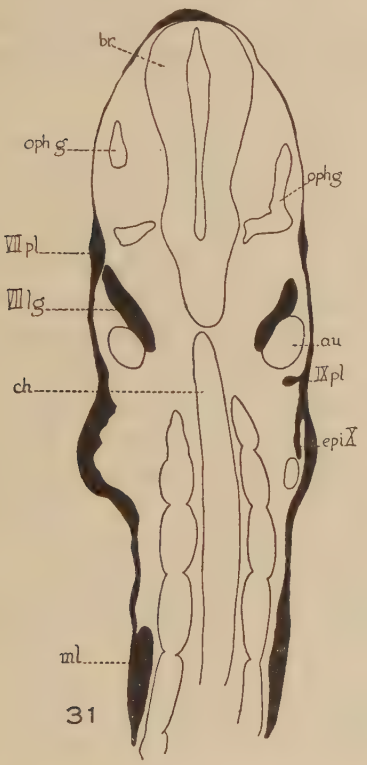
The lateral-line primordium in the lower portion of the hyomandibular cleft has increased in depth and at the same time has slightly lengthened. Its upper extremity lies along the posterior border of the anlage of the balancer (fig. 7).

The epibranchial placode of IX lies in the same position and still has a narrow contact with the placodal cells of the visceral sensory ganglion of IX. Over the first and second branchial clefts the epibranchial placodes of X, now continuous as a single elongated placode, are contributing large numbers of cells splitting off en masse to the visceral sensory ganglion of X lying ventrally to the vagus lateral-line ganglion (fig. 31).

Along the posterior border of the ear and slightly above the epibranchial placode of IX appears another rounded placode, which is the primordium of the anterior portion of the occipital group of lateral-line sense organs. It is in close contact with the IX lateral-line ganglion (fig. 31). Posterior to this and above the first branchial cleft is another rounded placode, which is the primordium of the posterior portion of the occipital group of lateral-line sense organs.

In the dorsal portion of the intersegmental ridge of the first and second somites is a rounded placode (fig. 7) connected by placodal cells from its anteroventral margin to the lateral-line ganglion of X, indicated by dotted line. This is the primordium of the dorsal body line of lateral-line sense organs. On a level with its ventral border an elongated club-shaped placode extends from the anterior border of the second somite to near the posterior border of the third somite. This is the primordium of the midbody line of sense organs and is connected by a small strand of cells at its anterior border to the posterior border of the lateral-line ganglion of X. Just below the anterior extremity of the primordium of the midbody line is a small rounded placode lying close to the posterior extremity of the lateral-line ganglion

near the dorsal extremity of the fourth branchial arch. This is the primordium of the ventral body line of sense organs. It is connected by a few placodal cells from its dorsal border to the vagus lateral-line ganglion.



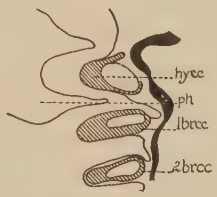


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Fig. 31 Frontal section, showing at stage 35 the IX lateral-line ganglion and placode anterior to epibranchial placode of X. Farther posteriorly is mid-body-line primordium. × 37.

Fig. 32 Frontal section, showing at stage 36 aggregation of crest cells on median surfaces of mesoderm of hyoid, first and second branchial arches. × 37.

It is at this stage in the developing embryo that all of the primordia of the lateral-line groups of sense organs are laid down in visible form in the ectoderm.

Stage 35+

Neural crest. With the exception of a more compact arrangement in the branchial and hyoid regions, there is but little change in the neural crest over the mesoderm of the branchial and hyoid arches (fig. 8). No crest cells are found over the dorsal and anterior borders of the auditory vesicle. Above the branchial arches and posterior to the auditory vesicle loose crest cells lie in the same relation to the branchial bands as in stage 35. In the mandibular region there has been but little change in the relation of the crest cells except for a steady decrease in the number of cells dorsal to the eye. Beyond this stage the neural-crest arrangement is obscured by the lack of color differentiation, by the further migration of crest cells to the median sides of the mesoderm of the arches and by the rapid growth of the external gills, so that further knowledge of crest-cell arrangement cannot be gained by dissection. The manner in which the crest cells assume their positions around the arches may be more clearly shown in the description of the process which takes place in stage 36.

Placodes. The primordia of the supra- and infra-orbital lateral-line sense organs, from their point of fusion posterior to the eye, curve over the dorsal and posterior borders of the eye, respectively, as narrow, consolidated, rod-shaped thickenings in the ectoderm (fig. 8). The supra-orbital primordium, when compared with its appearance in stage 35 (fig. 7), shows a more decided change in its shape than does the infra-orbital primordium. Posterior to their point of juncture, the primordium of the hyomandibular lateral-line sense organs extends ventrally along the upper portion of the hyomandibular cleft as a short rod similar in character to that of the supra- and infra-orbital primordia. The primordium of the ventral hyomandibular group of lateral-line sense organs now extends farther ventrally from the posterior border of the base of the balancer in the lower portion of the hyomandibular cleft and its rod-shaped placode has somewhat increased in depth. In addition to the primordia shown in the previous stage (fig. 7), there is now a primordium of the mandibular group of lateral-line sense organs extending as a short ectodermal thickening along the ventral border of the base of

the balancer fused with the dorsal extremity of the ventral hyomandibular primordium.

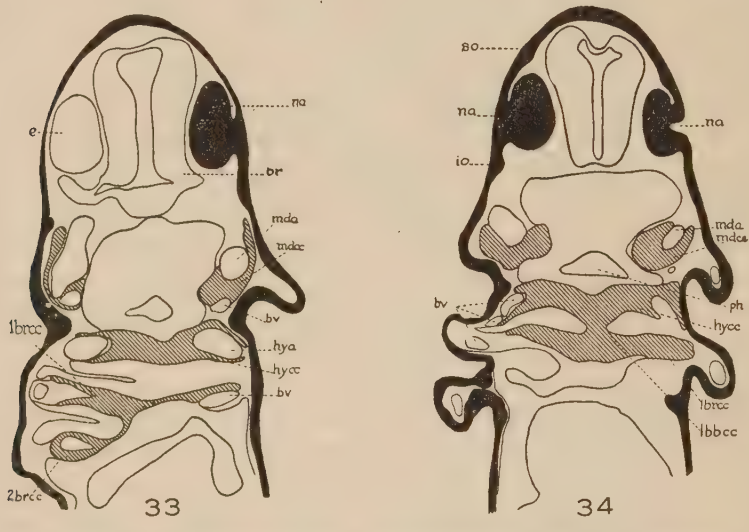
The epibranchial placode of VII still persists in contact with the visceral sensory ganglion of VII where many mitotic figures can be seen. The epibranchial placodes of IX and X still persist and a few cells appear to be splitting off to the visceral sensory ganglia.

The two primordia of the occipital group of lateral-line sense organs have increased in size and their close contact to the IX and X lateral-line ganglia gives them the appearance of contributing cells to those ganglia. The primordium of the dorsal body line of sense organs now appears as an elongated placode extending posterodorsally from the middle of the second somite to the middle of the third somite, connected at its anterior end by a slender lateral-line nerve to the posterior extremity of the vagus lateral-line ganglion whose position is indicated by dotted line. The midbody lateral-line placode is an elongated club-shaped structure extending from the middle of the fourth somite to the posterior border of the seventh somite. It is connected from its anterior border by a slender lateral-line nerve to the vagus lateral-line ganglion in common with the lateral-line nerve of the dorsal body line. The ventral body line is represented by a small ovoid placode which lies on the posterior border of the fourth branchial arch. It is connected dorsally to the posterior portion of the vagus lateral-line ganglion by a small lateral-line nerve.

Stage 36

Neural crest. Since stage 35, there has been a more rapid migration of the crest cells to the median border of the arches where they accumulate in large numbers (fig. 32). As one studies the sections farther ventrally, this becomes more apparent, and it can be seen that the process is carried on at the expense of the crest cells on the lateral, anterior, and posterior borders of the arches where they lie in a thin layer, or where, in the case of the lateral borders, they are occasionally entirely lacking. Within the aggregation of crest cells on the median borders of the arches

may be seen many mitotic figures indicating that there still is a rapid increase in the number of cells as well as a migration. Up to this point the crest cells have shown characteristic fine yolk granules in their cytoplasm which was an aid in differentiating them from the surrounding tissue. Now the yolk granules have become very small or have been largely absorbed within the crest-cell aggregations. In many places the mesoderm of the branchial



Figs. 33 and 34 Frontal sections, showing at stage 36 and 37–38, respectively, large numbers of crest cells at median and posterior borders of mesoderm of mandibular arch and fusion in the midline of crest cells of hyoid and first branchial groups to form the first basibranchium. ×37.

arches is being hollowed out by the formation of developing blood vessels.

If the sections are followed ventrally it can be seen that the crest cells of the hyoid arches unite in the midventral line in front of the pericardium. The first branchial group, after it has united with the second branchial group (fig. 33), joins the crest cells of the first branchial group and from the opposite side attempts farther ventrally to continue with the posterior border of the fusion of the hyoid crest cells. The third and fourth branchial crest-cell groups have not yet joined at their ventral extremities.

As one studies the ventral portions of the mandibular and maxillary processes, it is evident that there is an abundance of crest cells, especially around the former (fig. 33).

Placodes. The supra-orbital primordium now extends as far ventrally as the dorsal border of the olfactory organ (fig. 9), while the infra-orbital descends to the posterior border of the olfactory organ. The hyomandibular primordium has now joined the dorsal extremity of the ventral hyomandibular primordium posterior to the balancer. The mandibular primordium extends anteroventrally, then mesially to the region of the lower jaw. The ventral hyomandibular primordium extends farther ventrally, then mesially where it begins to take an anterior direction upon the ventral surface of the lower jaw. Posterior to the auditory vesicle, four separate placodes now mark the positions of the primordia of the occipital group of lateral-line sense organs. The two anterior placodes of this group lie close to the posterior border of the ear, while the two posterior lie above the base of the third external gill; the more ventral of the latter is much elongated in the anteroposterior direction.

The dorsal body-line primordium has extended as far as the seventh somite, followed by a thin lateral-line nerve upon which the anlagen of two sense organs have appeared. The primordium of the midbody line is a very much elongated placode which extends over about seven and one-half somites as far as the sixteenth somite. Along its lateral-line nerve appear the anlagen of five sense organs. The ventral body line can be seen descending behind the external gills anterior to the limb region connected by a slender lateral-line nerve.

The epibranchial placode of VII still has a slight contact with the distal end of the visceral portion of VII. No other epibranchial placodes are found to be giving off cells to the ganglia at this stage.

Stage 37–38

Neural crest. The aggregations of crest cells along the median borders of the visceral arches now show a more compact arrangement. So dense is the tissue that it appears to be composed

mainly of nuclei with but little cytoplasm. The density increases as one studies the sections farther ventrally. The few yolk granules which are present are similar in size to those of the smaller ones in the mesoderm of the arches and many mitotic figures can still be seen among the crest cells. The fusion of the median ends of the first branchial groups with those of the hyoid in the midventral line furnishes an illustration of the more compact arrangement of the cells (fig. 34).

As one follows the sections dorsally, the median ventral extremities of the branchial groups on each side are seen to approach each other at successive levels. It is now quite obvious that the branchial groups have taken on the form of the early branchial cartilaginous skeleton. The dorsal extremities of the branchial groups are continuous with loose crest cells, which possibly linger behind to form connective tissue, although the rapid differentiation of the surrounding mesoderm prevents any accurate determination of this. Contiguous to the neural-crest aggregations the blood-vessel spaces can be seen permeating the mesoderm of the arches, the circulation of blood in the mesoderm of the arches having been established in stage 36–37. The crest cells are more loosely arranged around the periphery of the mandibular arch—a possible indication that they are giving rise to connective tissue. Over the maxillary process the crest cells are followed with great difficulty owing to the fact that in front of, as well as behind the stomadaeum there are many loose mesenchyme cells. The crest cells have already made their way into the balancer, as has been shown by Harrison ('21).

Placodes. Since the epibranchial placodes no longer appear in the ectoderm, further description of the placodes will be confined to the primordia of the lateral-line system (fig. 10). The supra- and infra-orbital primordia extend farther ventrally, passing respectively, around the anterior and posterior borders of the nasal organ. They then pass medially in anticipation of the formation of the sense organs on the upper jaw around the external nares. The hyomandibular primordium has increased slightly in length and passes to near the posteroventral border of the balancer. The mandibular and ventral hyomandibular primordia have extended

further medially than in the previous stage described. The occipital group is still represented by four primordia, which are becoming elongated as they approach each other. The dorsal body line has progressed posteriorly as far as the twelfth somite, along the path of which have appeared the anlagen of a varying number of sense organs. The midbody line has progressed caudally as far as the twenty-seventh somite. As it reaches a point just above the anal region it curves dorsally a short distance, then caudally where it travels along the upper border of the somites of the tail. Along its course have developed many anlagen of sense organs. The ventral body line has descended behind the external gills anterior to the limb region to the ventral border of the mesoderm of the limb bud where it begins to bend caudally.

Stage 39

Neural crest. Since the previous stage the aggregations of neural crest have become still more compact (fig. 35) and may now be regarded as the procartilage of the visceral skeleton. Only a few crest cells can be identified outside of the procartilage region, although, as has already been suggested, many of the loose cells around the maxillary and mandibular groups may be of crest-cell origin and may be forming connective tissue. The procartilage still contains many small yolk granules and also many mitotic figures. The position of the mesoderm of the branchial arches is now occupied by blood vessels and anlagen of branchial muscles, while in the position of the mandibular and hyoid mesoderm are the anlagen of mandibular and hyoid muscles.

Stage 42

Neural crest. In stages 40–41, of which no sections have been figured, the only perceptible changes in the procartilage are the gradual disappearance of yolk granules, the decrease in the number of mitotic figures, and the steady increase in the density of the cellular arrangement. In stage 42 true cartilage is laid down as well-defined, solid bars in the positions formerly occupied by the procartilage (fig. 36). The yolk granules have entirely disappeared from the branchial cartilages.

A study of the early branchial skeleton up to this stage shows conclusively that so far the cartilages have their origin in the neural crest. However, in the case of the second basibranchial, which is the last cartilage of the visceral skeleton to appear, stage 42 shows more convincingly than any of the previous stages that this cartilage is of mesodermal origin. The caudal extremity of

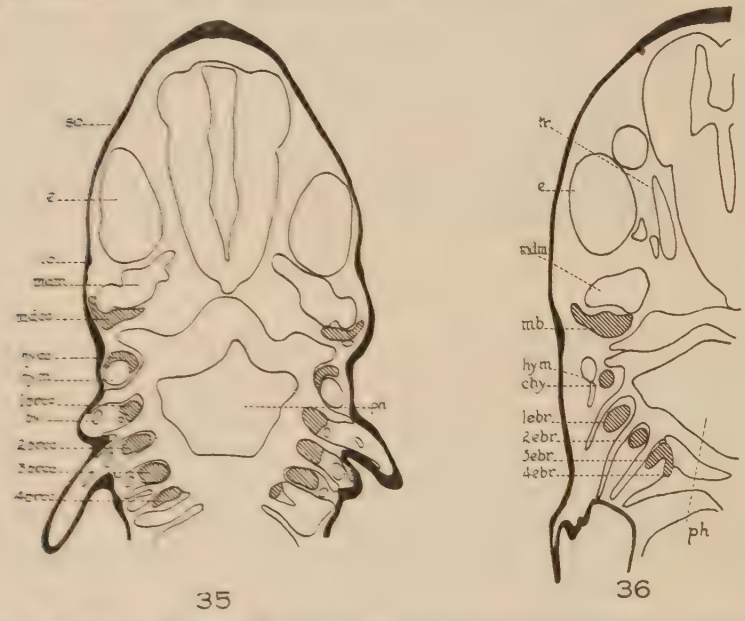


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Fig. 35 Frontal section through mesoderm of visceral arches at stage 39, showing positions of their procartilages. × 37.

Fig. 36 Frontal section through the cartilages of the arches at stage 42 when true cartilage is laid down, showing the same positions of the cartilages occupied by aggregations of crest cells. × 37.

the first basibranchial is attached to mesoderm which continues ventrally toward the pharynx. Out of this mesoderm along the anterior part of the pericardial chamber is formed the second basibranchial. Its distal half lies ventral to the level of the other cartilages. The mesoderm continuous with its distal extremity is the anlage of the thoracicohyoideus muscles, while the mesoderm continuous with its proximal or attached extremity is the anlage of the geniohyoid muscles. It has not developed cartilage

cells at this stage and its large characteristic mesodermal yolk granules (fig. 37), which are retained for a long time, make a sharp contrast with the other branchial cartilages, which now contain practically no yolk granules.

Stage 46+

Lateral-line groups of sense organs. The subsequent growth of the lateral-line primordia is merely a matter of the development

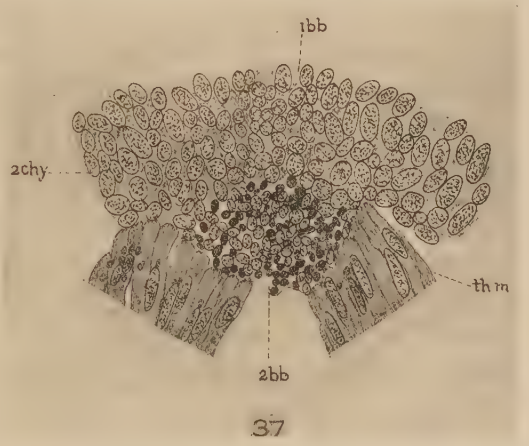


Fig. 37 Frontal section at the level of first basibranchium, showing at stage 42 formation of second basibranchium from mesoderm near anterior wall of pericardial chamber. × 250.

of sense organs out of the ectodermal thickenings as they are laid down in stage 37–38 (fig. 10).

The general distribution of the lateral-line sense organs has been described in a number of amphibians. Descriptive figures are shown by Kingsbury ('95) of the distribution of different groups of sense organs connected with the lateral-line system in eight groups of amphibians. However, two figures (figs. 11 and 12) have been included in this paper to show the groups of sense organs developed from the primordia of stage 37–38 as they are finally laid down in a 13½-mm. larva of Amblystoma punctatum.

The groups of sense organs may be described in the following manner: 1) *the supra-orbital*, a group usually arranged in a double line passing dorsal to the eye then ventral to a region median to the external nares; 2) *the infra-orbital*, a group sometimes in a single, sometimes in a double row, passing ventral to the eye to a region on the upper jaw ventral to the external nares; 3) *the occipital*, an approximately triangular group of variously arranged organs with a short line of four or five sense organs extending caudally as far as the region above the third external gill; 4) *the hyomandibular or postorbital*, an irregularly arranged group, usually a double line associated with the infra-orbital passing caudally and ventrally from the latter to the ventral side of the head; 5) *the mandibular*, a single line of sense organs extending along the side of the lower jaw from the symphysis back to the ventral extremity of the hyomandibular group and giving off, at the angle of the jaw, a branch of two or three sense organs, sometimes called the angular group, extending dorsally to the infra-orbital line; 6) *the ventral-hyomandibular group*, a double line of sense organs on either side, beginning at the symphysis of the jaw passing median to the mandibular group to meet the latter at its posterior extremity, where it gives off a single line of seven or eight sense organs which passes directly mesially, but does not join with the corresponding branch of the opposite side; 7) *the midbody line*, a single line of sense organs extending from a region above the proximal end of the limb behind the external gills to the region above the level of the anus, where it bends upward to pass along the upper border of the somites to the end of the tail; 8) *the dorsal body line*, a single line of sense organs extending from behind the dorsal end of the third gill along the upper border of the somites, where it joins the midbody line in the region above the anus; 9) *the ventral body line*, a single line of sense organs curving around the ventral border of the fore limb and extending to the ventral border of the hind limb.

In this morphological study, in which critical stages have been described, it is obvious that at the time of the closure of the neural folds (stage 21) no definite placodes can be accurately located, unless it be a slight indefinite thickening dorsal to the

eye. The ectodermal thickening over the dorsal extremity of the hyomandibular cleft as well as the longitudinal ectodermal thickening seems to have no significance except in so far as it is the result of a change in contour of the underlying tissue. As early as stage 25 (fig. 3), however, the placodes of lateral-line ganglia of VII and X can be identified, while all the epibranchial placodes can be located at stage 26–27 (fig. 4). The primordia of all the groups of the lateral-line sense organs are established at stage 35+ (fig. 8), and out of this pattern the sense organs are laid down in the skin.

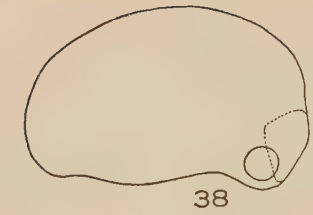
EXPERIMENTAL

All the operations were confined to the right side of the embryos and consist of two groups: *a*) extirpating the placodes in the ectoderm, followed by grafting into the wound indifferent ectoderm taken from another animal of the same age and, *b*) removing the neural crest either by scraping it loose or by removing it along with the upper half of the neural canal from the anterior border of the third somite to the anterior extremity of the brain above the eye.

Where indifferent ectoderm was transplanted into denuded areas the grafts were removed from animals which had previously been stained in the jelly capsule with Nile-blue sulphate (Detwiler, '17). This made possible daily observations upon the early growth and position of the implanted tissue.

The mortality of operated animals was lowered by keeping them during the three or four days following the operation in a glass chamber, around which cool tap-water ranging from 11° to 15°C. was constantly running. This retarded wound healing at first, but no disturbances were observed to affect the results of the experiments.

In stage 25 the auditory placode becomes marked externally as a small round pigmented spot a little below an elevation in the region of the hind-brain (fig. 3). This may be used almost invariably as a landmark in orienting operations upon the placodal regions in this and early embryos. Since there is no external evidence of the pigmented spot previous to this stage, the eleva-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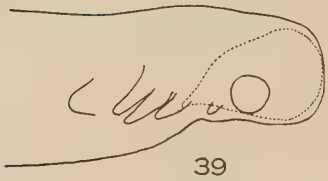
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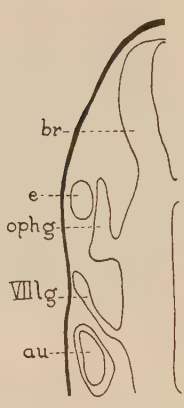
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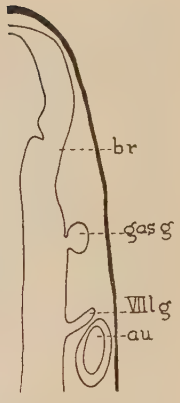
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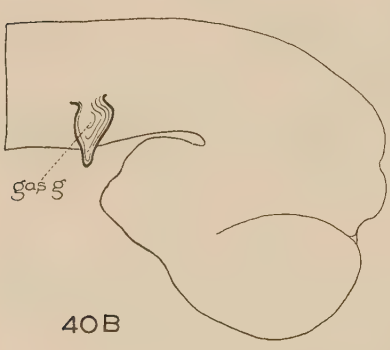
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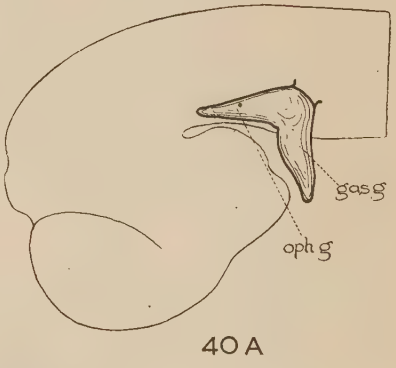
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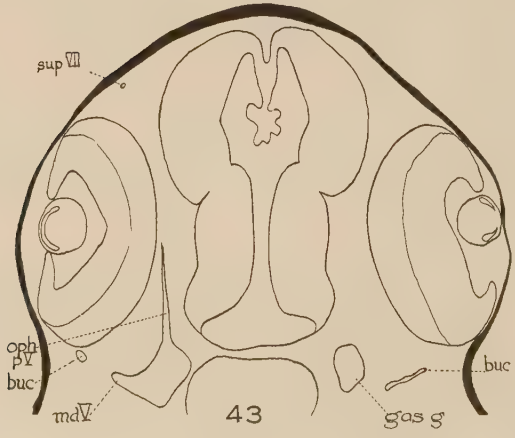
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tion in the region of the hind-brain lends itself as a landmark in orienting similar operations upon embryos from the closure of the neural folds to stage 25 (figs. 1 and 2).

A. Extirpation of placodes

1. Removal of ophthalmic placode. A triangular piece of ectoderm, including skin dorsal to the optic vesicle, was removed in the manner shown in figure 38 and indifferent ectoderm was grafted upon the denuded area. Such areas of ectoderm were taken from embryos varying in stages from 23 to 26.

A typical picture of the extension of the implanted blue ectoderm may be obtained from figure 39. Gradually the blue area extends ventrally until it includes the ectoderm over the eye and the regions anterior and posterior to it, always showing the more extensive migration towards the ventral half of the mandibular arch and towards the first external gill. As a result of this, the blue ectoderm several days after the operation comprises an area far greater in extent than the original extirpated area. After seven or eight days the Nile-blue sulphate gradually disappears and the transplanted area can often be located by its scarcity of pigment—a characteristic of the ventral ectoderm of older embryos. The same condition of the extension of blue implanted ectoderm may be seen in many other cases (figs. 48, 49, 55, 63). This may be largely due to a migration of the super-

Fig. 38 Showing triangular piece of ectoderm, indicated by dotted line and middorsal line which was excised in order to remove the ophthalmic placode at stage 23. × 10.

Fig. 39 Camera-lucida drawing of the same individual a few days after operation, showing extent of migration of implanted ectoderm stained in Nile-blue sulphate. × 10.

Fig. 40 a and b Showing, respectively, lateral views of a reconstruction in the trigeminal region of the normal and operated sides. Only a small gasserian ganglion appears on the operated side. × 50.

Figs. 41 and 42 Frontal sections of the specimen reconstructed in figures 40 a and b comparing the normal and operated sides at levels of the root of V. × 37.

Fig. 43 Frontal section of specimen killed nineteen days after operation, showing on right side only the gasserian portion of V after the ophthalmic placode has been excised. × 37.

ficial cells of the ectoderm, for the deeper-lying sense organs in the intruded area are undisturbed. However, this is a subject for further investigation.

A specimen killed eight days after operation shows a complete absence of the ophthalmic ganglion and the ophthalmicus profundus V nerve (figs. 40 a and b). The size of the ophthalmic ganglion of the normal side of this individual is very large (fig. 41), and a comparison between it and the region of the root of the V on the operated side (fig. 42) renders a striking picture. The crest cells concerned in the formation of the visceral skeleton along the region of the mandibular arch are approximately normal in amount. This condition indicates that if any of the crest cells had been disturbed there has been a regeneration, and ample opportunity has been offered to contribute to the formation of the ganglion if such were their function. This individual also shows a diminution in the size of the gasserian ganglion as illustrated by figure 40 a. Another individual killed nineteen days after a similar operation at stage 23 shows no ophthalmicus profundus V nerve and the small ganglionic mass which does appear is apparently concerned only with the gasserian portion (fig. 43).

An individual which had been operated upon at stage 26 shows nine days later a very small ganglion represented by possibly no more than ten cells (fig. 44). The only representative of the ophthalmicus profundus V nerve appears in but one section and extends anteriorly as a slender nerve fiber. During stage 26 cells are being given off from the ophthalmic placode, and it is quite possible that a few of the placodal cells remained in the wound and later gave rise to the remnant of the ganglion which appears in this section. The gasserian ganglion is also smaller in this individual than is the normal ganglion of the left side.

An individual which was operated upon at stage 23 shows a small ophthalmic ganglion in an abnormal position (figs. 45 a and b). This ganglionic mass lies median to the eye and somewhat dorsal to the optic nerve. It is connected to the brain by a slender group of fibers which pass back through the gasserian ganglion into the brain. At the lower anterior portion of this

ganglionic mass there arises a slender nerve which sends a few cutaneous fibers to the skin over the anterodorsal portion of the eye. It then follows over the dorsal border of the nasal organ, in the region of which it gives off cutaneous fibers. In the distribution of its fibers this nerve issuing from the anteriorly placed ganglion simulates the ophthalmicus profundus V nerve. A

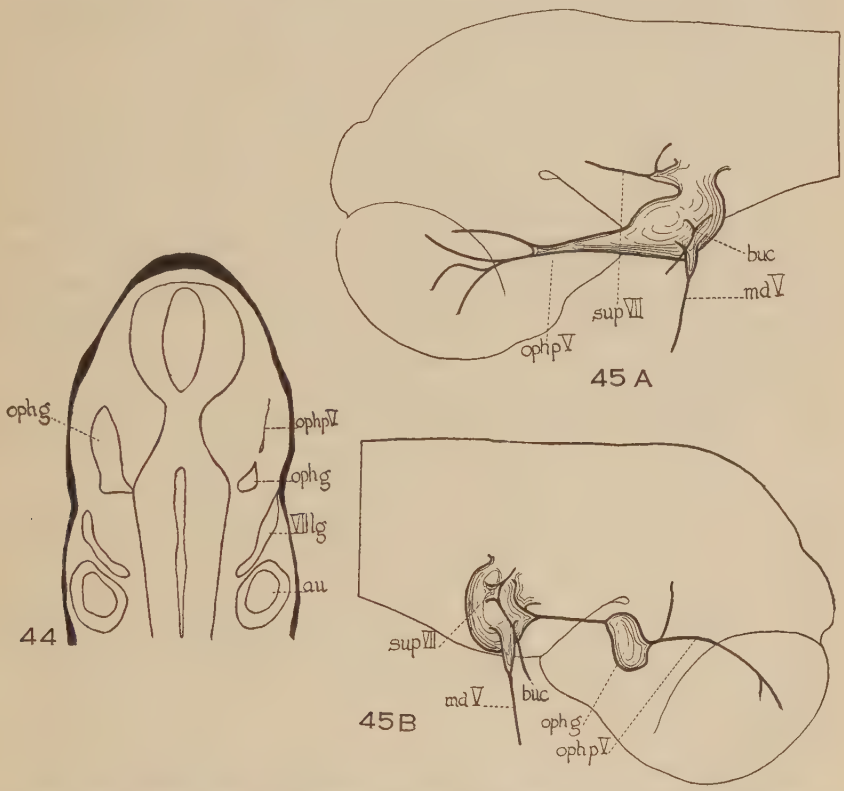

Fig. 44 Frontal section, showing relative difference in size of the ophthalmic ganglia on normal and operated sides. Part of the ophthalmic placode was possibly not removed. × 37.

Fig. 45 a and b Lateral views of a reconstruction, showing, respectively, normal and operated sides in the region of V and VII. The posterior portion of VII ganglion is not shown. The operated side (fig. 45 b) shows a small ophthalmic ganglion displaced anteriorly and lying above the optic nerve. It is connected posteriorly with the V and VII complex by a long slender root a few fibers from its dorsoposterior border. A small ophthalmicus profundus V nerve extends from its anteroventral border. × 50.

study of the records of the growth of the transplanted blue ectoderm of this individual shows that not all of the ectoderm concerned in the formation of the ophthalmic ganglion was eliminated from the ectoderm in the region anterodorsal to the optic vesicle.

A control operation was made on several specimens in which the ectoderm was excised as in the usual operative procedure and replaced and allowed to heal into its normal position. When these specimens were sectioned they showed perfectly normal ophthalmic ganglia, indicating that if there had been any disturbance of the crest cells in the trigeminal region during the operation it did not, in itself, affect the formation of the ganglion.

A large number of individuals in which the excised area containing the preauditory placode and supra-orbital primordium was removed along with some of the ectoderm above the optic vesicle, show a similar displacement of the ophthalmic ganglion. Among these cases where the embryos were preserved within a few days after the operation ganglionic masses of cells lie close to the ectoderm. Their posterior ends become attenuated and no connection to the brain is discernible. The older individuals which were sectioned show cutaneous fibers issuing from the ganglionic mass comparable in their distribution to the fibers from the ophthalmicus profundus V nerve as in the case already cited (fig. 45 b).

It seems quite evident from the above results obtained that the formation of the ophthalmic ganglion is largely if not entirely dependent upon the placode in the ectoderm above the optic vesicle.

2. Removal of gasserian placode. A rather extensive rectangular piece of ectoderm was removed, including all the ectoderm around the dorsoposterior quadrant of the eye. An incision was made beginning at a point on the hyoid arch at a level with about the middle of the eye and passing dorsally some distance in front of the auditory placode to a level about the middle of the latter. The incision was then extended anteriorly parallel with the mid-dorsal line to a point above the middle of the eye. From this point it was carried ventrally to a little above the middle of the

eye, from which it was extended posteriorly to the incision on the hyoid arch. The ectoderm thus outlined was very carefully removed and the wound was covered by a graft of blue indifferent ectoderm.

A specimen killed six days after operation at a time when the ophthalmic and gasserian ganglia have just fused at the point of the entrance of the trigeminal root into the brain shows on the operated side a somewhat smaller gasserian ganglion. The frontal sections show that the ganglion in its anteroposterior diameter varies little in the region near its root from that on the normal side, but as the ganglion is followed ventrally it soon becomes attenuated in the region near the upper posterior border of the eye, while on the normal side the mandibular nerve does not appear until the ganglion reaches the level with the middle of the posterior border of the eye. The diminution in the size of the gasserian ganglion is therefore represented mostly by a shorter dorsoventral axis. A large portion of the ophthalmic ganglion is displaced and lies near the anterodorsal portion of the eye. A group of fibers connect it to ganglionic cells which lie on the anterodorsal border of the gasserian ganglion. The crest cells over the mandibular arch are apparently normal in amount.

Two other specimens killed the same number of days after operation show the same results. In these cases, however, there appears to be a slightly smaller number of crest cells over the mesoderm of the mandibular arch.

A control operation was also made on several specimens, in which the ectoderm was excised in the usual manner and then replaced and allowed to heal in its normal position. Sections of these specimens show perfectly normal gasserian and profundus ganglia.

It has already been shown (figs. 40 b and 43) that when the ophthalmic placode was removed, including considerable ectoderm from the posterodorsal region of the eye, the gasserian ganglion was smaller than on the normal side. Among the cases, described in another section of this paper, in which placodes of VII were removed there appear two cases in which there is apparently no gasserian ganglion present (fig. 56 b).

It seems quite evident from these results that the ectodermal adhesion of the early gasserian ganglion is in the nature of a small placode which contributes cells to the formation of that ganglion.

3. Removal of preauditory placode and the supra-orbital primordium. In this type of operation the rectangular area of

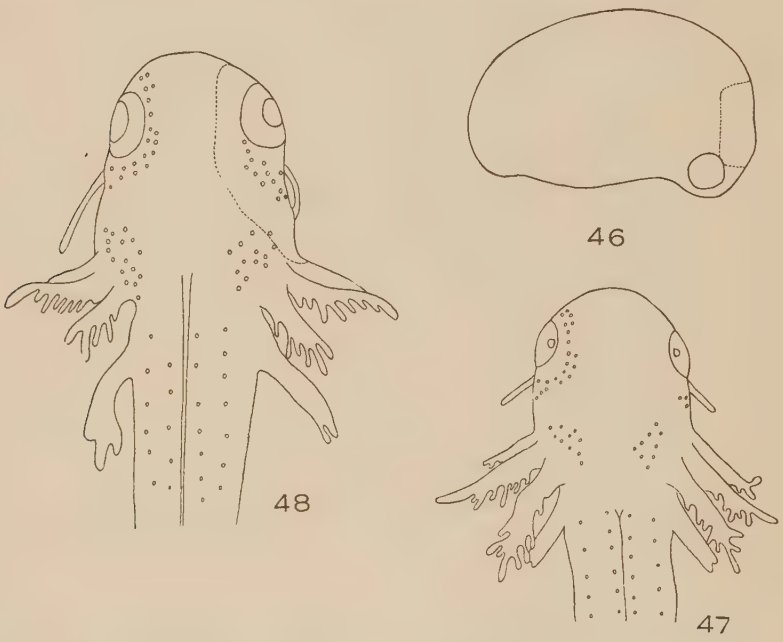

Fig. 46 Camera drawing showing area of ectoderm excised in removing preauditory placode and supra-orbital primordium. Outlined by means of dotted lines and the middorsal line. × 10.

Fig. 47 Camera drawing, showing absence of the supra-orbital group of sense organs after removal of an area of ectoderm as shown in figure 46. × 10.

Fig. 48 Camera drawing, showing only three or four supra-orbital lateral line sense organs when not all of the early preauditory placode is removed during stage 21. The lightly pigmented area of transplant of indifferent ectoderm at time animal was killed is shown by dotted line. × 10.

ectoderm excised is outlined in figure 46. Indifferent ectoderm from the ventral side of another animal of the same age which had been previously stained with Nile-blue sulphate was grafted into the wound. This operation was confined to stages under stage 25.

Due to the inability to locate exactly the position of the auditory placode, especially in stage 21, it was often included wholly or in part in the excised ectoderm. Among such cases where the auditory vesicle was not entirely absent, it appeared as a small rudimentary vesicle near the skin.

An examination of specimens which were treated in the manner just described shows in eight cases no supra-orbital group of lateral-line sense organs (fig. 47), and consequently no ophthalmicus superficialis VII nerve. The operations in seven of these cases were performed at stage 23 and in one case about stage 21. The auditory vesicle appears as a rudiment in six cases, while in the other cases it is entirely absent. The larger portion of the lateral-line ganglion of VII which supplies nerve fibers to the supra- and infra-orbital groups of sense organs is represented in these cases by a very small ganglion. The infra-orbital line of sense organs with its corresponding nerve was never absent in this type of operation and it received its fibers from the small remaining portion of the above-mentioned lateral-line ganglion.

In a few cases where a small portion of the placode was, presumably, not entirely extirpated, the VII lateral-line ganglion is smaller than on the normal side and a small line of sense organs is the only representative of the supra-orbital line (fig. 48). In some cases a sensory line was represented by small, poorly developed organs and the ophthalmicus superficialis VII is so slender that it can be followed with great difficulty and then only when most favorably stained. Such cases show diminution in the size of the VII lateral-line component which supplies the supra-orbital and infra-orbital sensory lines. Throughout all these cases in which the supra-orbital sensory line is represented in part or in its entirety the ear is present and apparently normal in size.

The hyomandibular, ventral, and mandibular groups of lateral-line sense organs were never disturbed when such an area of ectoderm was removed. The VII lateral-line component of the ganglion supplying nerves to these groups was always normal. It is quite apparent that no other portions of the VII ganglion are lacking.

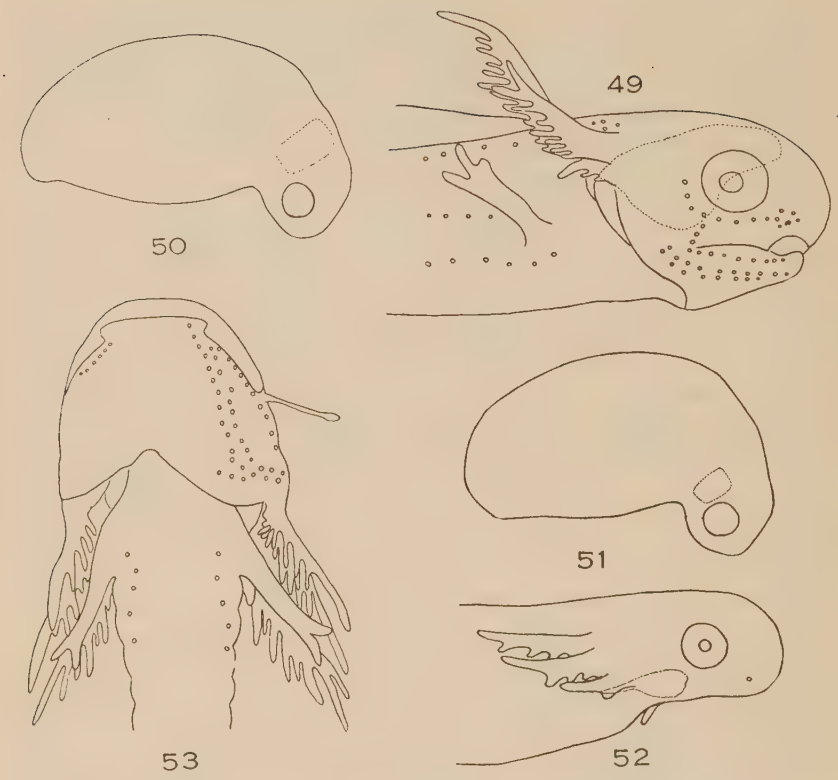

Fig. 49 Camera drawing, showing absence of hyomandibular group of sense organs after removal of an area of ectoderm shown in figure 50. × 10.

Fig. 50 Camera drawing, showing by dotted line an area of ectoderm removed at stage 26 in which was included the primordium of the hyomandibular group of sense organs as well as epibranchial placode of VII and supra-orbital primordium. × 10.

Fig. 51 Camera drawing, showing by dotted line an area of excised ectoderm in which was included the primordium of the ventral hyomandibular group of sense organs and part of the primordium of mandibular group. × 10.

Fig. 52 Camera drawing, showing by dotted line position of implanted blue ectoderm a few days after operation in same individual shown in figure 51. The balancer is absent on operated side. × 10.

Fig. 53 Camera drawing twenty-one days later of the ventral side of embryo shown in figure 51. On the operated side there is only a small portion of the mandibular group of sense organs, while the ventral hyomandibular group is entirely absent. × 10.

4. Removal of the infra-orbital, hyomandibular, ventral hyomandibular and mandibular primordia. A number of trials were made upon embryos under stage 26 to remove separately, if possible, the primordia of the infra-orbital and hyomandibular groups of lateral-line sense organs on the side of the head by excising small segments of ectoderm behind the upper posterior border of the optic vesicle. These attempts proved to be fruitless, for in all cases the ingrafted tissue failed to suppress their development and complete regeneration of the two primordia took place. However, among the cases where an area of ectoderm including placodes of the VII ganglion was removed there appears one case, operated upon at stage 26, in which the hyomandibular group of sense organs is entirely absent, although the infra-orbital group is intact as well as the groups of sense organs on the lower side of the jaw (fig. 49). The area removed (fig. 50) also includes the epibranchial, the preauditory, and supra-orbital placodes. This seems to indicate that the infra-orbital and hyomandibular groups have separate primordia.

A few operations were made in an attempt to remove the primordia of the sense organs on the under side of the jaw. The most successful case appears in an individual operated upon at stage 25. The area removed is shown in figure 51, one day after operation, and it includes a small amount of ectoderm anterior and posterior to the lower portion of the hyomandibular cleft. Several days after operation (fig. 52) the area of the transplanted indifferent ectoderm may be seen at a lower level than its original position and the migration of its posterior portion extends into the anteroventral border of the first external gill. The balancer is lacking on this side. Twenty-one days after operation this individual reveals no ventral hyomandibular group of sense organs on the right side of the ventral portion of the lower jaw (fig. 53). However, there is a remnant of the mandibular group in the form of a few small sense organs along the anterolateral border of the lower jaw. This may possibly be an indication of the existence of a separate primordium for the mandibular group of sense organs.

5. Removal of the epibranchial placode of VII and surrounding ectoderm. The area of the extirpation in this type of operation

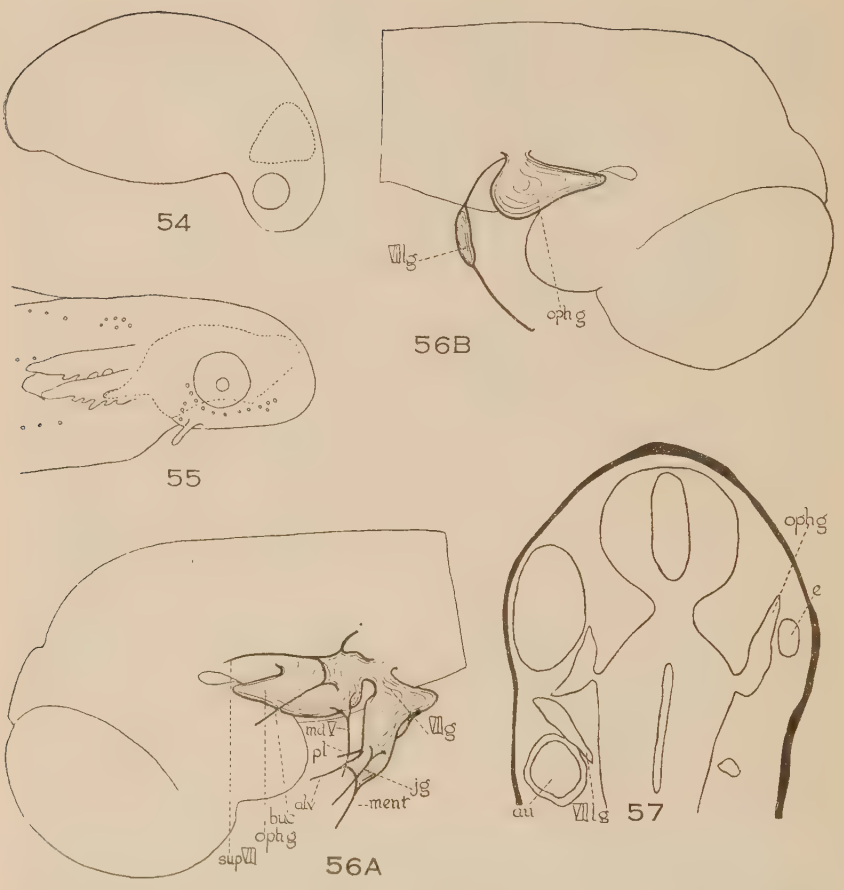

Fig. 54 Camera-lucida drawing, showing by dotted line extirpated area of ectoderm one day after operation. The operation was done at stage 25 and included auditory and preauditory placodes as well as epibranchial placode of VII. × 10.

Fig. 55 Camera drawing of embryo shown in figure 54 twelve days later, showing extent of area of implanted blue ectoderm and absence of supra-orbital line. × 10

Figs. 56 a and b Lateral views of a reconstruction of the V and VII ganglionic complex of animal figured in 55, showing respectively, the normal and operated sides. The operated side shows a small VII lateral-line ganglion as only representative of VII. There is also no gasserian ganglion on operated side. × 50.

Fig. 57 Frontal section, showing only a small VII ganglion after removal at stage 26 of an area of ectoderm similar to that shown in figure 54. × 37.

is outlined from a case one day after operation (fig. 54). The operation was done at stage 25 and the extirpated area included the auditory placode as well as ectoderm anterior to it, although its anterior extremity did not include as much ectoderm as in the cases of the removal of the preauditory placode and supra-orbital primordium already described. The ventral extension of the extirpated area reached as far as the lower portion of the hyoid arch and in a few cases the mesoderm of the hyoid arch was exposed farther posteriorly than is the case in figure 54. The extension of the blue area at the time of killing, twelve days later, is shown in figure 55. All the groups of sensory lines are present except the supra-orbital group, although the sense organs which do appear on the right side of the head and lower jaw are not as prominent as on the normal side.

A reconstruction of the VII ganglionic components reveals only a small portion of this ganglion on the operated side (figs. 56 a and b). It lies lateral and slightly posterior to the ganglion of V and is elongated in the dorsoventral axis. As near as can be determined, a very slender root of fibers enters the brain near the posterior border of the V. The lower extremity of this small ganglion receives slender nerve fibers from the regions of lateral-line sense organs on the side of the head and the ventral side of the lower jaw. No palatinus, alveolaris, nor cutaneous portions of the jugularis nerves can be found on the operated side as are shown on the normal side (fig. 56 b). The ceratohyoid cartilage is slightly smaller on the operated side. The ganglionic portion of V in this individual seems to be concerned only with the ophthalmicus division. Another specimen which had a similar area removed during stage 25 was killed nineteen days after operation, and it also shows the same results.

A similar operation upon an embryo at stage 26–27 shows a small VII ganglion (fig. 57). The fibers from it seem to be lateral-line fibers to the sense organs on the lower part of the head. There is distinctly no palatinus VII, and whether the other components are entirely lacking or not cannot be exactly determined, for the animal was killed within a few days after the operation, and the small VII ganglion on the operated side has not developed

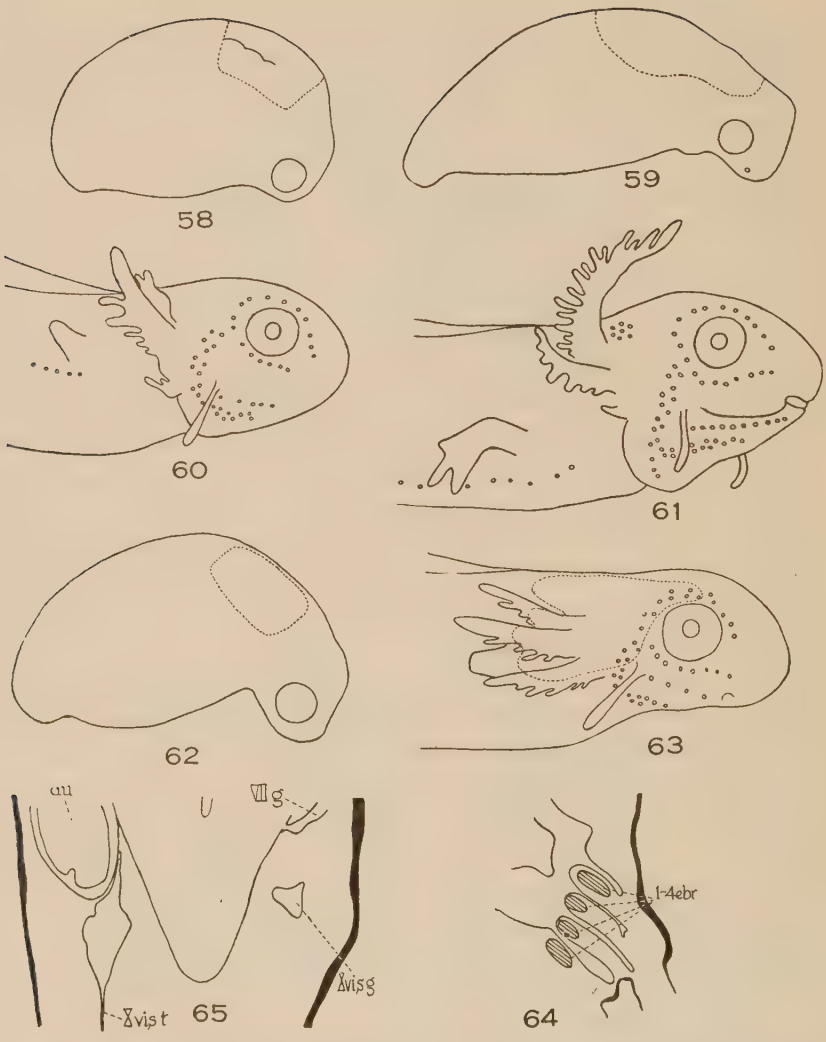

Fig. 58 Camera drawing, showing in dotted line and middorsal line in the area of ectoderm removed at stage 23 in excising postauditory lateral-line primordia and epibranchial placodes of IX and X. The auditory placode was also included. × 10.

Fig. 59 Camerad rawing, showing in dotted line the area of blue implanted ectoderm two days after operation, not including all the ectoderm along the posterior border of the gill swelling. × 10.

Fig. 60 Camera drawing ten days after operation, showing presence of a ventral body line of sense organs when posterior border of gill swelling is not removed as shown in figure 59. × 10.

sufficiently to say definitely how much is really lacking. The gasserian ganglion is much smaller in this individual. The ceratohyoid procartilage is normal on the operated side. In all cases where such an area as just described was removed there always appeared the same results, viz., a very small ganglion whose slender fibers supplied sense organs on the side of the head and lower jaw was the only representative of the VII ganglion.

6. Removal of epibranchial placodes of IX and X and postauditory lateral-line primordia. The operations were done around stages 21 and 26 and involved the removal of varying amounts of ectoderm posterior to, and often including, the auditory placode. The most satisfactory results were obtained in removing ectoderm outlined in the manner shown in figure 58.

Whenever the upper posterior border of the gill swelling was not removed (fig. 59) there appeared a small ventral body line of sense organs (fig. 60), although the dorsal and midbody lines were entirely absent. This occurred in many specimens and indicates, as shown in the descriptions of the normal material, that there is a separate placode for the ventral body line.

In a number of cases the occipital group of sense organs occurs (fig. 61), although the auditory vesicle was removed. The anterodorsal portion of the gill swelling was not well covered according to an observation of the blue ectoderm two days after the operation. The occipital or ventral body lines may persist after the operation, but only after operations done around stage 21. It appears that the area capable of regenerating these primordia is diffuse at this early stage.

Fig. 61 Camera drawing, showing only a few sense organs of occipital group when not all ectoderm is removed over anterodorsal portion of gill swelling. No dorsal nor midbody line of sense organs is present. × 10.

Fig. 62 Camera drawing, showing in dotted line twelve hours after operation at stage 26 the area of ectoderm removed, including all postauditory lateral-line primordia and epibranchial placodes. × 10.

Fig. 63 Camera drawing of embryo figured in 71, showing thirteen days after operation the extent of blue ectoderm and absence of postauditory lateral-line sense organs. × 10.

Fig. 64 Frontal section of the embryo shown in figure 63, showing normal branchial arches. × 37.

Fig. 65 Frontal section through similar level on either side, showing the small visceral ganglion of X on the operated side. × 37.

When the operation is done at about stage 26 and an area is removed as shown in figure 62, about twelve hours after operation, no postauditory primordia of the lateral-line sense organs appear. Only lateral-line sense organs anterior to the ear may be found. The extent of the blue area is marked out by dotted line (fig. 63).

An examination of certain typical specimens in this type of operation does not show such an extensive lack of ganglionic material as found in the regions of V and VII when all the crest cells are present.

The case illustrated in figure 63 shows normal branchial arches (fig. 64) and certain small ganglionic masses of IX and X. There is a very small X visceral ganglion (fig. 65). There are no first nor second branchial trunks. From the posteroventral portion of this ganglion a slender trunk comparable to the visceral trunk of the vagus passes posteroventrally alongside of a branchial blood vessel behind the anlage of the branchial bars to the posterior portion of the pharynx where its fibers are lost against the side of a blood vessel. There are no lateralis fibers in the visceral trunk because there are no lateral-line ganglia in the IX or X complex. A small visceral ganglion of IX lies below the level of X visceral ganglion at a lower level than on the normal side. Through it pass a few motor fibers which are given off to branchial muscles. A slender branchial trunk passes to the lateral side of the anlage of the levator arcus branchialis primus muscle to which it appears to give off a few motor fibers. Here it is lost in the first branchial arch and gives no evidence of containing sensory fibers. No special visceral fibers pass anteriorly toward the pharyngeal region.

Another specimen killed eighteen days after operation shows a few poorly developed sense organs in the occipital region which are supplied by a few nerve fibers from a very small IX lateral-line ganglion (fig. 66). A few lateralis fibers come from a small X lateral-line ganglion to innervate the posterior sense organs of the occipital group. The visceral ganglion of the vagus is somewhat smaller than on the normal side and gives off a visceral trunk which passes through a small ganglion on the median side of the fourth branchial arch. This ganglion seems to be a portion of

the vagus lateral-line ganglion which has possibly been formed by the ventral body-line primordium. The visceral trunk passes from this ganglion to the region of a small blood vessel, alongside of which it continues toward the posterior portion of the pharyngeal wall, where it is lost. Lateralis fibers also pass from this ganglion down to the ventral body line which is present for a short distance in this individual. Very slender first and second branchial trunks extend from the lower portion of the visceral

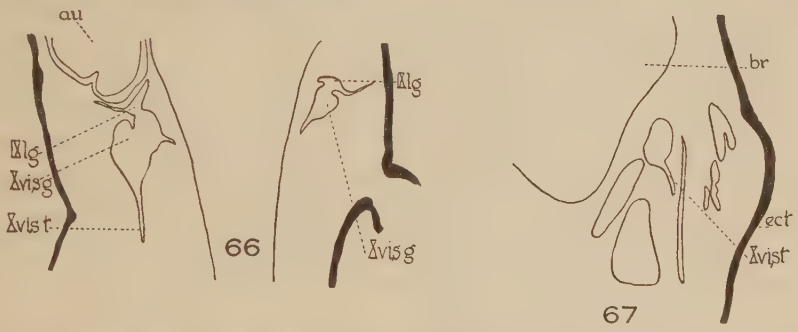

Fig. 66 Frontal section, showing the small visceral ganglion of X on operated side in front of which lies the IX lateral-line ganglion giving off fibers to occipital group of sense organs. A similar level on normal side shows a large IX and X ganglionic complex. The epibranchial placodes of IX and X were removed in this individual, but the occipital and ventral body-line primordia were not removed. × 37.

Fig. 67 Frontal section of a specimen from which epibranchial and lateral-line placodes of IX and X were removed, showing the X visceral ganglion and visceral trunk of X issuing from its posteroventral portion. It contains no lateralis fibers. × 37.

ganglion into the second and third branchial arches, but do not appear to carry any sensory fibers. The visceral ganglion of IX emerges close to the root of VII from a root which lies at a lower level than on the normal side. It gives off a trunk ventrally which passes into the first branchial arch running along close to a blood vessel for some distance until it is finally lost against the branchial muscles of this arch. This specimen also gives no evidence of containing visceral fibers in the sensory system.

Another specimen killed twenty-two days after operation shows all cartilages present, but slightly smaller in their dorsal portions while their lower portions are normal. Posterior to the ear there is but one occipital organ which is innervated by a single fiber coming from a small group of ganglionic cells—possibly the X ganglion. This ganglionic mass is very small and fused against a small vagus ganglion which is apparently only a visceral ganglion. Motor fibers may be seen issuing from the region of this ganglion to the branchial muscle. From the ventral side of this vagus ganglion a few short fibers extend to the region of blood vessels, where they are lost. Only the first branchial trunk of the vagus can be found and it contains only motor and general visceral fibers. The visceral trunk of the vagus ganglion may be seen issuing from the posteroventral portion of the ganglion (fig. 67). It carries no lateralis fibers, but is composed of general visceral fibers which are lost in the posterior region of the pharynx. The vagus lateral-line ganglion is entirely absent. No visceral ganglion of IX can be found.

These cases are typical of the findings from a study of a large number of individuals and the results seem to show that whenever the lateral-line placodes are removed no lateral-line ganglia are present, and when the ectoderm is removed from so large an area as to include the epibranchial placodes of IX and X and surrounding ectoderm no sensory fibers of the cutaneous and the special visceral system are present, while on the other hand the general visceral component is derived from the neural crest.

B. Removal of neural-crest cells

1. Contribution to mesodermal tissue. The neural crest was exposed to view by a longitudinal incision in the ectoderm beginning at a point below the middle of the third somite in the anterodorsal border of the pronephros and extending cephalad through the ectoderm of the dorsal third of the eye to the midline. The posterior extremity of the longitudinal incision was extended dorsally to the middorsal line, slanting slightly caudad. The ectoderm thus outlined was flexed dorsally hinging on the middorsal line.

An attempt to eliminate the neural-crest cells was made in several ways. By means of scissors and a fine spear-point needle the neural-crest cells were scraped away from the wall of the neural canal and, according to age, also carefully lifted off the mesoderm upon which they had migrated. The ectoderm was then turned back into its normal position, where it was weighted with glass rods and silver wires in the usual manner and allowed to heal. This treatment on one side only did not eliminate the possibility of the neural crest either from the sources of its origin on the operated side or from cells of similar groups wandering over from the unoperated side. Therefore, a series of embryos immediately after this operation was put into a cool chamber to retard development, and ten or twelve hours later the normal left side was similarly treated. In this type of double operation, however, the absence of normally developed crest cells makes a comparative study difficult.

A study of a large number of the first type of operation in which the crest cells were scraped away from one side only shows a large percentage in which regeneration of the crest cells had taken place, while many of the specimens which had undergone the double operation proved to be very oedematous in the gill region and unfit for study. Many of these individuals also showed complete regeneration of the neural crest.

In order to insure against the regeneration of the neural crest, a more extensive operation was performed. The ectoderm was flexed back in the manner already described and, after the neural crest had been carefully scraped away, the upper half of the neural tube on the right side was removed from the anterior border of the third somite to the anterior extremity of the brain above the eye. The number of cases available was considerably diminished by the high mortality attending the presence of such a large wound—only fifteen out of one hundred and fifty surviving. The high mortality was partially overcome by diminishing the area of operation by one-half. The ectoderm was flexed back upon the middorsal line so as to expose the crest cells over the branchial region. The crest cells along with the upper half of the neural tube from the anterior border of the third

somite to just in front of the ear were removed. In cases where it was advantageous the crest cells upon the mesoderm of the hyoid arch were included among the tissue that was removed. The number of recoveries from this kind of operation was far in excess of the number of deaths.

The neural crest over the mandibular and hyoid regions was removed along with the upper half of the neural tube from the posterior border of the ear to above the anterior border of the eye. The mortality in this type of operation far exceeded the recoveries. The ectoderm lying above the optic vesicles is very narrow, and when it is cut loose along its ventral border it soon shrinks toward the middorsal line, so that when the neural canal is removed the anterior extremity of the wound is often poorly covered. The healing is often incomplete at the anterior extremity of the head. The hole remaining, however small, is responsible for a large percentage of the deaths.

Among the group of embryos from which the neural crest has been removed by scraping there appear many cases which show varying degrees in the diminution of the size of the external gills. A very few showed slight deficiencies in the branchial cartilages.

The most favorable results were obtained from the specimens from which the neural tube was removed along with the neural crest. Among those cases in which this method was used to eliminate the crest cells over the branchial region and occasionally over the hyoid region there are recorded a number of specimens killed at various stages in development after the operation.

Case 1. The operation was done at about stage 26 and the embryo was killed three days later. It had not developed very rapidly as it remained in the cool chamber up to the time of killing. Frontal sections through the mesoderm of the arches on the normal side show that the crest cells are completely surrounding them. A section at the level of the middle of the optic cups (fig. 68) shows a complete absence of crest cells on the mesoderm of the second branchial arch on the operated side. On the median side of the mesoderm of the first branchial arch only a few crest cells appear, which are continued in a few sections fur-

ther ventrally and then cease. They may be remnants of crest cells which adhered to the outer border of the mesoderm at the time of operation. However, their number is insignificant compared to the amount of similar cells on the normal side. The crest cells around the mesoderm of the hyoid arch are also considerably less in amount than on the normal side.

Case 2. The operation was done at the same stage as in the previous case and the specimen was killed after six days. A frontal section (fig. 69), although somewhat oblique, shows in the

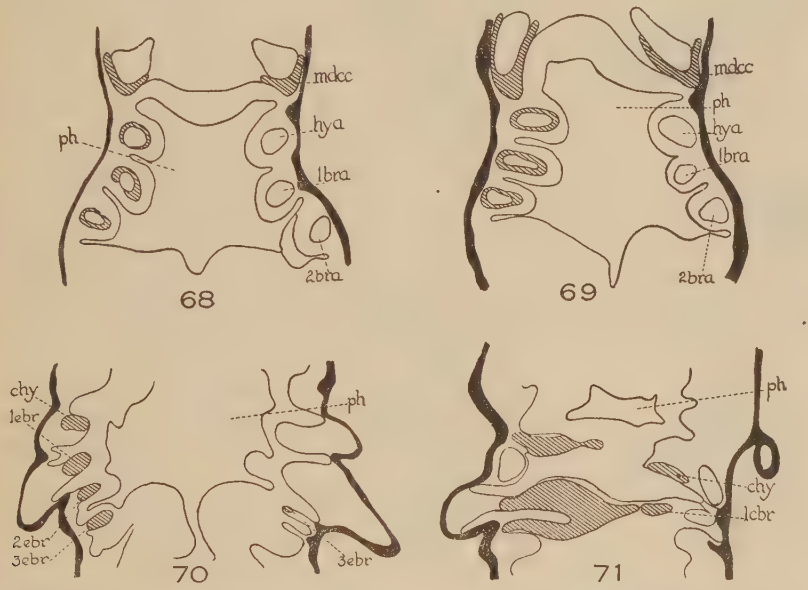

Fig. 68 Frontal section through the mesoderm of hyoid, first and second branchial arches, showing, three days after removal of crest cells, no crest cells on right side. × 37.

Fig. 69 Frontal section of specimen killed six days after removal of crest cells in hyoid and branchial regions, showing a distinct lack of crest cells on right side around the mesoderm of hyoid and first and second branchial arches. × 37.

Fig. 70 Frontal section of specimen killed nine days after removal of crest cells over the hyoid and branchial regions, showing only three procartilage cells in the third branchial arch. × 37.

Fig. 71 Frontal section at a lower level in the same specimen as in figure 70, showing only a few procartilage cells in the hyoid and first branchial regions. × 37.

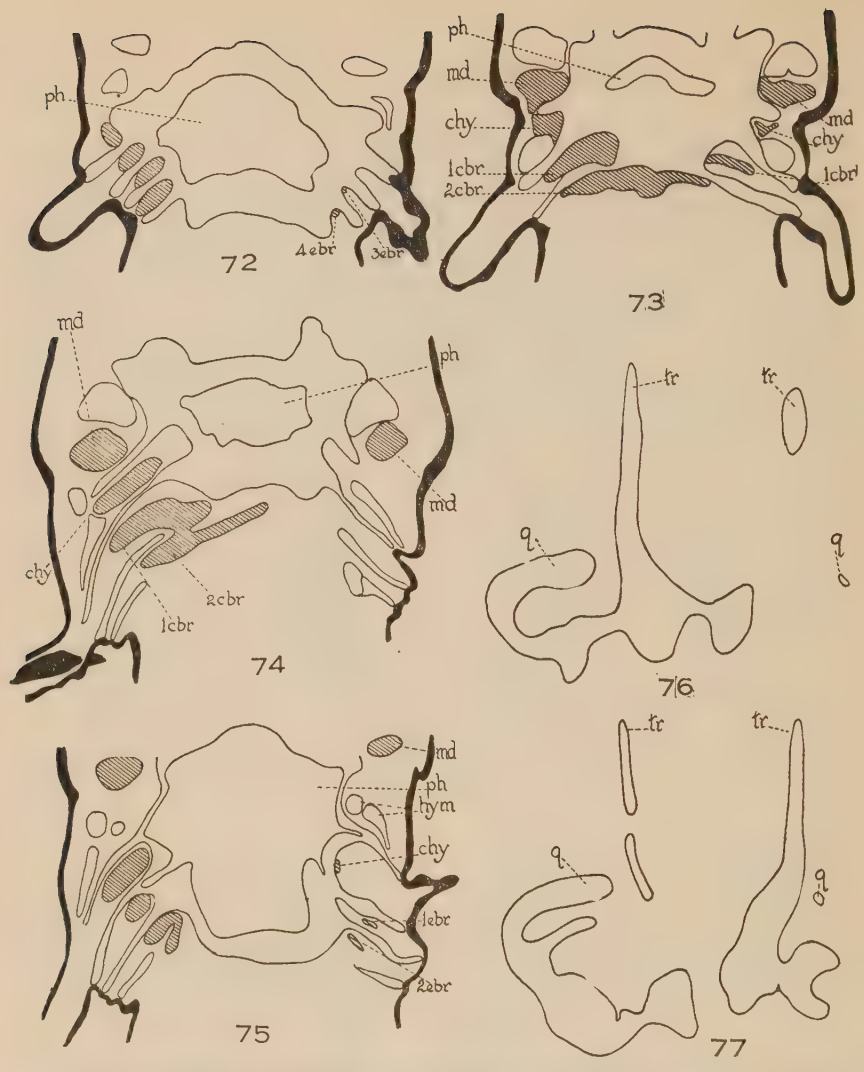

Fig. 72 Frontal section through procartilages of the arches of a specimen killed fourteen days after operation, showing only a few procartilage cells in the third and fourth branchial arches. × 37.

Fig. 73 Frontal secton at a lower level in same specimen as in figure 72, showing only a few procartilage cells in the hyoid arch and a small number in first branchial arch on operated side. × 37.

Fig. 74 Frontal section of specimen killed seventeen days after removal of crest cells from hyoid and branchial regions, showing absence of ceratohyal and first and second ceratobranchial cartilages on the operated side. × 37.

Fig. 75 Frontal section of a specimen killed eleven days after operation, in which an attempt was made to remove all the crest cells, showing only rudiments of cartilages in the hyoid, first and second branchial arches. The quadrate is smaller than normal. × 37.

Figs. 76 and 77 Frontal sections at two levels in the same specimen, showing the small size of the right quadrate cartilage. × 37.

region of the mesoderm of the hyoid and the first and second branchial arches a distinct lack of crest cells on the operated side, while on the normal side they are abundant and still surround the mesoderm of the arches.

Case 3. A similar specimen was killed nine days after operation, when it had reached about stage 37. The external gills at this stage have become prominent finger-like processes, but have not developed branches. A frontal section of this specimen (fig. 70), although obliquely cut, shows to even more advantage the comparison of the two sides, for the level of the operated side is lower where there would be normally an abundance of procartilage-forming cells. There are but three crest cells in the region of the third branchial arch, while the others lack cells of this kind altogether. This is the condition in sections above this level. The crest cells in the hyoid region are also noticeably less in amount than on the left side. At a lower level (fig. 71) the difference is more striking, for the hyoid, the first, and part of the second branchial regions have very few cartilage-forming cells compared with the abundance on the normal side. Loose connective tissue in the external gills on the operated side is also subnormal in amount.

Case 4. This specimen was killed fourteen days after the operation, when it had reached about stage 39. The gills on the operated side are smaller than on the normal side and their connective-tissue cells are less numerous. Compared with the normal side, there are only a few procartilage cells in the third and fourth branchial arches (fig. 72). When followed farther ventrally (fig. 73), the few loose cells on the right side increase but slightly in number and always remain much more loose in arrangement as they approach in the midline the normal compact procartilage. The procartilage in the hyoid region is also much less in amount.

Case 5. This specimen was killed seventeen days after operation and also shows a decided diminution in the size of the gills on the operated side. A frontal section shows well-formed branchial cartilages on the left, while on the right a few cells form a small cartilage only in the third gill arch. When followed

farther ventrally, very small rudiments of the second, third, and fourth branchial cartilages appear, which, after they approach each other at their ventral extremities, become completely lost and do not approach in the midline the cartilages of the normal side. The hyoid cartilage is completely absent. The first and second ceratobranchials and the ceratohyoid are absent on the operated side (fig. 74). The first and second basibranchials are present.

A specimen in which all the crest cells were included in the operation along with the upper half of the neural tube was killed eleven days after operation, and it shows many deficiencies in the visceral skeleton. Very small first, second, and third epibranchial cartilages are found on the operated side (fig. 75); they soon disappear as the sections are followed ventrally. The quadrate in this and other sections is smaller than on the normal side. The hyohyal, ceratohyal, and all the ceratobranchial cartilages are entirely absent. The mandible is smaller on the right side. The first basibranchial cartilage is present but small and lies toward the right of the midline, while the anlage of the second basibranchial is apparently normal. No change can be seen in the anterior portions of the trabeculae.

A similar attempt was made in another specimen, but due to incomplete removal of crest cells in the branchial region regeneration had taken place. However, seventeen days after operation it showed externally a diminution in size of the right side of the lower jaw. A section at a level with the lower border of the ear and the optic nerve shows a remnant of the quadrate very small (fig. 76) when compared with figure 77, a similar level on the left side. At the level of the lower border of the right eye the quadrate cartilage appears again for a short distance and extends toward the articular end of the mandible. As the sections are followed farther ventrally, the mandibular cartilage on the right side is seen to be smaller than on the normal side.

Another specimen was operated upon at stage 26 and killed twenty-one days later. At this stage it is impossible to remove successfully all of the crest cells which have migrated down over the mesoderm of the mandibular arch. The external gills and

lower jaw on the operated side showed an abnormal development. A dissection of this individual was made after the cartilages had been stained with methylin blue and shows (fig. 78) only three small branchial cartilages on the right side while the first and second basibranchial cartilages are present, but abnormally placed to the right of the midline. The quadrate and mandibular cartilages are somewhat smaller (figs. 79 and 80). There is not much difference in the size of the anterior portions of the trabeculae.

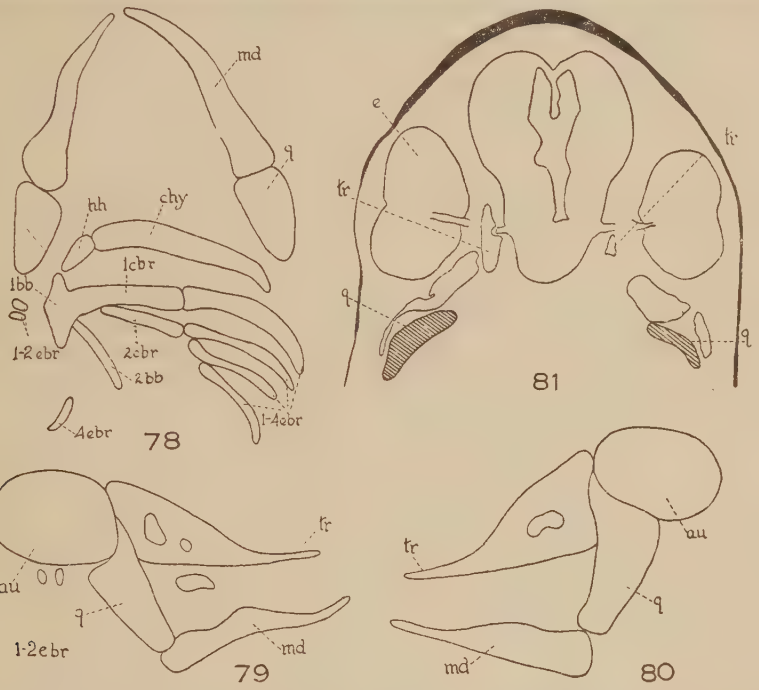

Fig. 78 Showing a camera-lucida drawing of a dissection of cartilages stained in methylene blue. Killed twenty-one days after operation. An attempt was made to remove all the neural crest in this specimen. Only three small branchial cartilages appear on the operated side. The first basibranchium is less developed on the right side, while the second basibranchium is a slender rod and displaced. The right mandibular cartilage also appears smaller than the normal. × 23.

Figs. 79 and 80 Camera drawings of lateral views of a dissection of specimen figured in 78, showing, respectively, operated and normal sides. The quadrate and mandibular cartilages are smaller on the operated side. × 23.

Fig. 81 Frontal section, showing in another specimen at the level of the optic nerves relative sizes of the quadrates when the crest cells have been removed at stage 24. × 37.

Another individual was operated on at stage 24 and the crest cells along with the upper half of the neural canal were removed from the hyoid and mandibular regions. There was evidently a regeneration of the crest cells, but the specimen showed certain deficiencies in the visceral skeleton. At a level with the optic nerves a section (fig. 81) shows a small quadrate cartilage on the operated side. In levels above and below this section the difference in the size of the two cartilages is even more striking. The anterior portions of the trabeculae show a difference in size. The anterior portion of the trabecula on the normal side extends somewhat beyond the level of the optic nerve, while on the operated side it extends only to the posterior border of the optic nerve. The ceratohyoid cartilage is poorly developed and joins with the first basibranchial posterior to its normal position. The mandibular cartilage in this individual also shows a deficiency in size.

It was found in the large number of operations made in removing the neural crest that the most favorable stage was around stage 26. However, this applies only to the branchial and hyoid regions. Here their removal is a very simple matter and no damage to the mesoderm need be expected, for the crest cells lie loosely upon the mesoderm and do not extend very far ventrally. In the mandibular region the elimination of crest cells is more difficult, for several factors are involved. From stage 23 to 26, when the regeneration of the neural crest is most persistent, a very rapid ventral growth takes place far in advance of the ventral proliferation of crest-cell groups in the hyoid and branchial regions. The crest cells lie tightly against the mesoderm of the mandibular arch, and in order to remove these cells considerable damage is caused to the mesoderm. The wound is very extensive ventrally, and the pulling of the cut edge of the ectoderm near the optic vesicle, accompanied by the large cavity made in the anterior portion of the brain, always resulted in the death of such individuals. It is hoped that by doing a large number of operations in this region a few specimens may survive which will show a complete removal of the crest cells over the trigeminal region.

An examination of the specimens done at the earlier stage shows a deficiency of the mandibular and quadrate cartilages, but not a complete absence. One case shows a deficiency in the anterior portion of the trabecula. In the branchial and hyoid regions it is quite evident that the branchial cartilages, the ceratohyal, the hyohyal, and the first basibranchial are derived from the wandering neural crest.

2. Contributions to ganglionic components. a. Contribution to V and VII. As already shown in the cases described, there is no condition in which the crest cells in the region of the trigeminus were entirely removed, although in the other regions they have been almost entirely eliminated.

A specimen operated upon at stage 25 was killed two days after operation and shows on the operated side a portion of the ophthalmic placode which is giving off cells, even though few crest cells may be found in this region. It lies close to the brain (fig. 82) while farther ventrally placodal cells from the posterior portion of the VII lateral-line placode are projecting medially to form the lateral-line ganglion (fig. 83). At its anterior portion it is contiguous with a small group of cells which belong to the gasserian placode. The epibranchial placode of VII at this stage is prominent, but has split off no cells on either side.

Although the crest cells have regenerated to a small degree in the trigeminal regions of two other specimens killed within a few days after operation, placodal cells may be seen contributing in large numbers to an ophthalmic ganglion. The condition of the ganglion is practically the same as on the normal side. One of the specimens shows a large well-formed ophthalmic ganglion (fig. 84), but the number of crest cells on the mesoderm of the mandibular arch farther ventrally is far less than on the normal side. Although there are but few crest cells on the mesoderm of the hyoid arch of this specimen, the VII lateral-line ganglion is present and normal (fig. 84). This level does not show the comparative size of the two ganglia. The visceral portion of the VII seems to be a little smaller than on the normal side.

Another typical case killed eleven days after operation shows deficiencies in the mandibular group of crest cells and an entire

absence of the ceratohyal cartilage. The ophthalmic ganglion is large and normal in size, although it is situated somewhat dorsal to its normal position (fig. 85). The portion of the lateral-line ganglion of VII which supplies the supra-orbital and infra-orbital group of sense organs also appears in this level and is normal in size as well as the rest of the lateral-line ganglion.

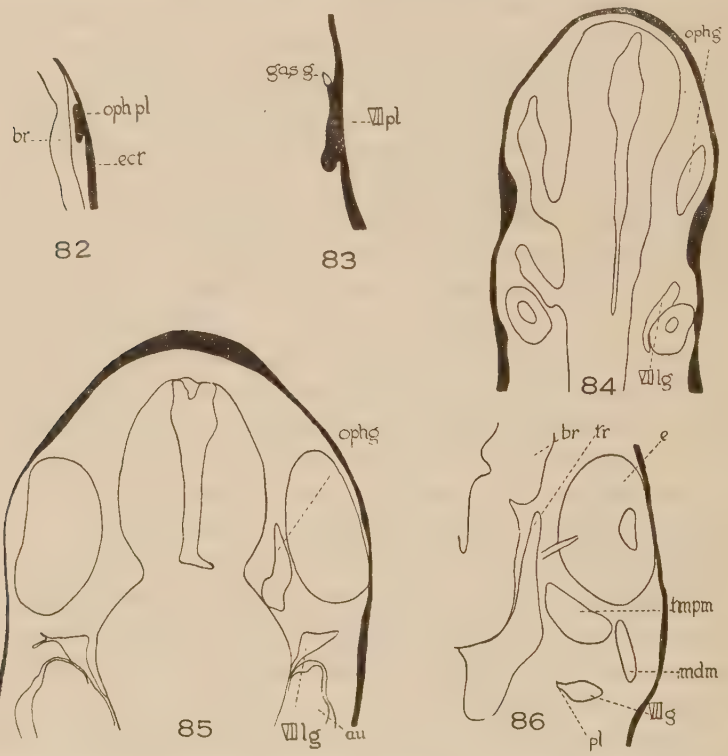

Fig. 82 Frontal section, showing the ophthalmic placode two days after the crest cells have been removed. × 37.

Fig. 83 Showing in the same specimen the posterior portion of VII lateral-line placode giving off placodal cells. At its anterior extremity is shown the contact of the gasserian ganglion with the ectoderm. × 37.

Fig. 84 Showing a normal VII lateral-line ganglion when crest cells have been removed from the hyoid arch. × 37.

Fig. 85 Frontal section, showing a large ophthalmic ganglion after crest cells had been removed at an early stage. × 37.

Fig. 86 Frontal section further ventrally in the same individual, showing the palatinus VII at the point of leaving the ganglion. × 37.

The gasserian ganglion is slightly smaller than on the normal side and lies somewhat separated from the ophthalmic ganglion. The visceral portion of VII is a little smaller and no definite alveolar nerve can be found. The special visceral or palatinus VII is present (fig. 86).

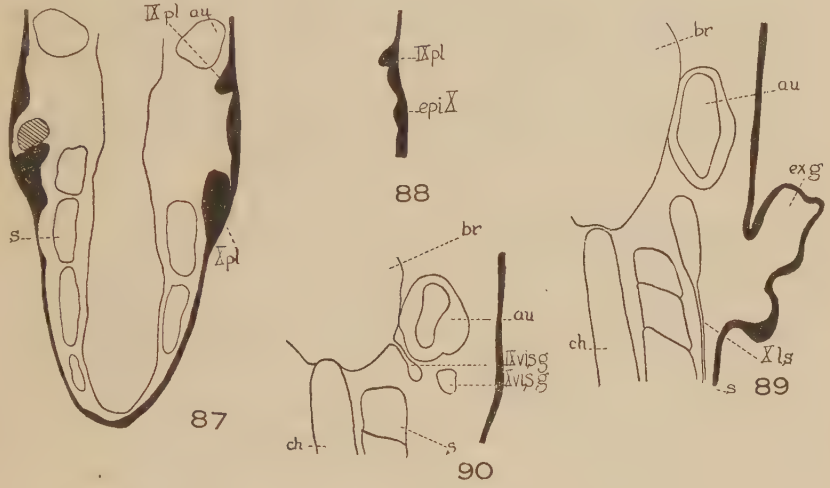

Fig. 87 Frontal section of a specimen killed three days after removal of crest cells over the branchial region, showing lateral-line placodes of IX and X forming ganglia. Between them in ectoderm lies the epibranchial placode of X. On the normal side lies a group of crest cells near the epibranchial placode of X, possibly forming a portion of visceral ganglion of X. × 37.

Fig. 88 Portion of a frontal section in a specimen killed a few days after removal of crest cells, showing normal epibranchial placodes of IX and X. × 37.

Fig. 89 Showing a large vagus lateral-line ganglion and ramus lateralis superior vagi in a specimen killed eleven days after an attempt had been made to remove all crest cells on right side. × 37.

Fig. 90 Showing in the same specimen the IX visceral ganglion. × 37.

b. Contribution to IX and X. A specimen killed three days after operation shows on the operated side a large lateral-line ganglion placode of IX just anterior to the epibranchial placode of X (fig. 87). On the anterior surface of the large vagus lateral-line placode are a number of loose placodal cells given off from the placode. No crest cells are found in the branchial region of the operated side. On the normal side anterior to the vagus

placode is a mass of crest cells near the ectoderm and epibranchial placode of X. These are crest cells which possibly give rise to a portion of the visceral ganglion, for when followed ventrally they lie close to the epibranchial placode of X.

A specimen killed five days after operation shows on the operated side a large normal lateral-line ganglion. The placode of the lateral-line ganglion of IX seems to be a little less advanced in development than on the normal side. Farther ventrally (fig. 88) loose masses of cells are being given off from the epibranchial placodes of IX and X and are not of crest-cell origin, for there are no crest cells in the branchial region of the operated side.

A specimen killed eight days after operation shows a large vagus lateral-line ganglion, in front of which is a portion of a small visceral ganglion. The ganglia are still made up of loose cells and nerve fibers from the visceral ganglia cannot be determined. Posterior to the auditory vesicle on the operated side is a small lateral-line ganglion which lies above the small visceral ganglion of IX which is connected with the epibranchial placode. Only a very few scattered crest cells appear on the median surface of the mesoderm of the branchial arches. A number of other specimens killed between eight and ten days after operation show small visceral ganglia, which are derived from the epibranchial placodes, and perfectly normal IX and X lateral-line ganglia. In these cases also there are only a few loose crest cells in the branchial region on the operated side.

A typical specimen killed eleven days after operation shows a large vagus lateral-line ganglion with the ramus lateralis superior vagi nerve extending posteriorly to innervate the body line of sense organs (fig. 89). The anterior portion of this ganglion is part of the visceral ganglion of X. From it pass slender fibers to the epithelium of the second external gill and also a number of motor fibers to the branchial trunk of the vagus can be seen in the second branchial arch. It gives off motor fibers to the branchial muscles, and when followed ventrally into the branchial arch it is lost near the epithelium on the pharyngeal side. There is no definite second branchial trunk. As the superior lateralis

vagi nerve leaves the ganglion, fibers are continued ventrally, as on the normal side, to form the visceral trunk of the vagus. A few motor fibers are given off along its path, and when followed farther ventrally it is finally lost near the wall of the pharynx. No ramus intestinalis from the visceral trunk can be determined. In the dorsal portion behind the ear a number of cutaneous fibers may be seen, along with the lateralis fibers, to innervate the skin. The visceral portion of IX may be seen at the lower border of the ear coming off from the root of IX which is at a lower level than on the normal side (fig. 90). The visceral ganglion is small and gives off no trunk to the first branchial arch, but ventral to the ear capsule it sends out a nerve which passes some distance along the median border of the internal ceratohyoid muscle, where it is followed ventrally until its fibers are lost against the wall of the pharynx. The only visceral fibers that can be identified on the operated side appear to be of the special visceral system. The other fibers which appear in the IX and X ganglionic complex on the operated side are of the lateralis and general cutaneous systems. In the ventral positions of the third and fourth branchial arches only very small rudiments of cartilages appear, which shows that very few crest cells remained which could have contributed to the visceral ganglia.

It appears from the study of the specimens described that the only contribution of the crest cells to the ganglionic complex of IX and X is to the general visceral component.

DISCUSSION

It has been shown in the study of early stages of Amblystoma embryos that extensive contributions from the lateral ectoderm take part in the formation of cranial ganglia, and the experimental analysis of the problem has shown how small a part the crest cells play in the formation of these ganglia. The facts which the experiments present lead to the conclusion that the general cutaneous system is derived entirely from placodes. In the trigeminal region there are two definite placodes concerned with the formation of the V; that in the case of the ophthalmic division is the larger, while that of the gasserian is the smaller and

of shorter duration and, therefore, difficult to follow. The earliest stage in which Coghill ('16) described the early contact of the ophthalmic ganglion with the ectoderm corresponds to about stage 34, i.e., a stage between those shown in figures 6 and 7. At the point of contact lies the placode which can be last seen at this stage. The actual contribution of placodal cells to the ganglion must be observed in earlier stages than this, at a time when crest cells are still very numerous in this region. This condition has been the factor which has caused investigators to overlook the placodal contribution. Coghill's observation that during this contact with the ectoderm the ganglion makes its connection with the brain adds further morphological evidence that the ganglion is of placodal origin. Judging from the many similarities in Amblystoma and Necturus, it is obvious that Platt was correct in assuming an ectodermal contribution to the ophthalmic ganglion, although she has confused the placodal and crest cells in this region and incorrectly interpreted part of these placodal cells as contributing to the 'mesectoderm.' The ectodermal cells which Goette ('14) describes being given off above the optic vesicles in Siredon (Amblystoma tigrinum) and Torpedo are likewise cells of the ophthalmic placode and not contributions to the wandering 'ectomesoderm.' It has been shown that when the ophthalmic placode is entirely removed in early stages of Amblystoma there is a complete absence of the ophthalmic ganglion and the ophthalmicus profundus V nerve (figs. 40 b and 43). Such cases always show but little disturbance of the crest cells in the trigeminal region. In control operations in which the ectoderm was removed in the usual manner and then replaced there appeared normal ophthalmic ganglia and nerves, which shows that the placode is necessary for the formation of the ganglion. When a small portion of the ectoderm over the eye is left a very small displaced ganglion is often found (fig. 45 b). In this respect it is similar to other partially removed placodes. On the other hand, when the ophthalmic placode is left intact and the crest cells have been disturbed as much as possible by an attempt to remove them, an apparently normal ganglion is present, and although there has been a regeneration of the crest cells

it shows that an extensive disturbance of the crest cells as early as stages 21 and 23 does not inhibit the growth of the ganglion.

As Coghill ('16) has already shown in Amblystoma, there is an early contact of the gasserian ganglion with the ectoderm. At the point of contact near the anterior border of the preauditory placode (figs. 21 and 26) is a small thickening in the ectoderm which can be followed only through stages 28 to 30. Although this condition has not been described in any other forms, it is quite possible that it does exist, but has been overlooked on account of its small size and short duration in a region where the crest cells are very abundant. Among the cases where the removal of the preauditory placode included ectoderm near the posterodorsal border of the optic vesicle there occurred two cases (fig. 56 b) in which there was no gasserian ganglion. When smaller areas of ectoderm were removed from the posterodorsal region of the eye there often occurred small gasserian ganglia. This was possibly due to the fact that not all of the placode had been removed. In one case where there was a deficiency in the crest cells on the mandibular arch after the crest cells had been removed a smaller gasserian ganglion was observed. In this case it seems quite possible in the light of the control and other operations that the gasserian placode was injured when the ectoderm was reflected at the time of operation.

The remaining portion of the general cutaneous system of the cranial nerves is to be found in the X. In the observations reported in this paper no definite distinction could be made in the early stages between the small general cutaneous and the visceral ganglia of X. Coghill ('16) has observed that during its early contact with the ectoderm, the cutaneous ganglion of X has no connection with the brain. However, when a large area of ectoderm was removed containing the epibranchial placodal regions of IX and X no definite general cutaneous fibers could be found. This leads one to conclude that the general cutaneous portion of the vagus complex is derived from the lateral ectoderm and the early contact of the small general cutaneous ganglion of X, which was described by Coghill, is the indication of a placode in the ectoderm which gives rise to that ganglion. This fact falls in

line with the results obtained in the removal of placodes in the trigeminal region and shows that the general cutaneous system is placodal in nature and not, as Landacre ('10) has suggested, of neural-crest origin.

From the results obtained in the series of experiments recorded in this paper there can be no doubt that the lateral-line sensory system in Amblystoma is derived entirely from placodes. The study of the preauditory placode shows that a large part of the lateral-line ganglion of VII is formed from this placode and from its anterior end arises the supra-orbital primordium of sense organs. The other lateral-line primordia in the head region are separate in origin as in Necturus and also contribute to the VII lateral-line ganglion. At no time is there any condition such as that described in Lepidosteus by Landacre and Conger ('13) in which the preauditory placode begins to disintegrate at the time when the first trace of the lateral-line primordium can be detected. It is quite possible that Landacre and Conger were misled in this interpretation of the preauditory placode, for, according to their description, it apparently arises very early, and although they describe no cells being given off from the placode, it seems probable that there may have been an early contribution which was unobserved.

In the case of the postauditory lateral-line primordia, the study of experimental as well as of normal material shows that the three trunk lines of sense organs have separate primordia, and in this respect Amblystoma is similar to Necturus. The experimental results show that the occipital group of sense organs appeared in a few cases where the ectoderm in the anterodorsal portion of the gill swelling (fig. 61) was not entirely removed, although the ear was entirely removed. This condition implies the independence of the occipital primordia from the auditory placode and also indicates in embryos close to stage 21 the ability of the ectoderm in the anterodorsal region of the gill swelling to give rise to occipital primordia. The complete removal of the postauditory lateral-line primordia was not only accompanied by the absence of the groups of sense organs, but by an entire absence of the lateral-line ganglia. When only a few sense

organs appeared, correspondingly small lateral-line ganglia were present which innervated those sense organs. No evidence can be obtained that crest cells contribute to the formation of lateral-line ganglia. The morphological studies are misleading in this respect, for in many cases the close arrangement and contact of the early crest cells and placodes make an interpretation of the exact contribution of the two kinds of cells difficult to understand.

In the study of the normal embryos the epibranchial placodes of VII, IX, and X could be located as early as stage 26–27 (fig. 4) and their contributions to ganglia could be followed up to stage 36. The observations of these placodes agree in many respects with Coghill as to the placodal relation and contribution to the visceral ganglia. The removal of epibranchial placodes was found to be accompanied by a distinct lack of gustatory fibers in VII, IX, and X with no apparent disturbance to the general visceral system. In this respect the experimental results agree with Landacre's explanation of the function of the epibranchial placodes in Lepidosteus, viz., that they give rise to special visceral ganglia of VII, IX, and X.

Goette ('14) expresses the belief that the epibranchial and lateral-line placodes form, with the crest cells, a syncytial mass of cells out of which ganglia and nerves are formed which later join themselves up with the brain. Studies of experimental and normal amblystoma embryos show that certain definite portions derived from placodes and crest cells, although they mingle with each other, maintain their identity and are not to be considered a syncytial mass at any time.

It has also been shown in Amblystoma that the neural crest originates from the dorsal portions of the contiguous surfaces of the neural folds at the time of the closure (fig. 13). These crest cells were followed by means of their difference in pigment and by the presence of fine yolk granules in their cytoplasm as they migrate ventrally over the mesoderm of the arches, always remaining separate from the ectoderm. The wandering mass of ectoderm is of crest-cell origin only and does not in Amblystoma receive any contribution from the ectoderm on the lateral surfaces of the head. Platt's descriptions of Necturus show clearly

that the positions of the proliferating lateral ectoderm correspond to placodal regions and since the migration of crest cells soon produces a scarcity of these cells in the dorsal region of the neural canal and an abundance of crest cells in the region where placodal cells are given off, a condition is brought about which would lead to a confusion as to the origin of the wandering 'mesectoderm.' Aside from the different interpretation in the origin of the 'mesectoderm' in Amblystoma the manner of the formation of the branchial cartilages with the exception of the second basibranchial agrees with the description which Platt ('97) gives of the branchial cartilages in Necturus. This is fully in accord with Landacre ('21). The branchial cartilages with the exception of the second basibranchial have been conclusively shown to have their origin in the neural crest. At the time when this skeleton begins to take on a cartilaginous appearance the first basibranchial extends a short distance posteriorly from the attachment of the ceratobranchial cartilages. This condition is somewhat misleading for it gives the appearance that the second basibranchial is a posterior outgrowth from the first basibranchial. However, such is not the case, for a study of embryos about stage 42 conclusively shows that the second basibranchial is formed out of mesoderm near the anterior wall of the pericardial chamber and that this cartilage retains large mesodermal yolk granules for a long time after the branchial skeleton from the neural crest has lost all of its yolk granules (fig. 37). Amblystoma in this respect agrees with Landacre's ('21) description of Plethodon. The experimental results show that some of the neural crest is incorporated in the connective tissue of the external gills as well as in the balancer, as Harrison ('21) has shown. How much more of the connective tissue in the branchial region is formed from the crest cells is impossible to determine at this time.

In the case of the mandibular and quadrate cartilages the experimental results, as already stated, did not show as conclusively as the findings from the study of normal embryos that they are derived from the neural crest, because of the difficulty in eliminating the crest cells from the trigeminal region. Nevertheless, the results do show that there was a decided diminution

in the size of those cartilages when the crest cells were removed. Although it was difficult to determine from the studies of normal embryos what became of the neural crest migrating over the dorsal and anterior margins of the optic vesicles, one case (fig. 81) in which the crest cells were removed in the trigeminal region seems to show conclusively that they form the anterior portions of the trabeculae. This is in accordance with the findings of Platt ('97) and Landacre ('21).

In no case do the crest cells enter into the formation of any part of the branchial musculature as described by Goronowitsch ('93). The musculature of the visceral arches is formed entirely from the mesoderm of those arches. No portion of the skull other than the anterior portion of the trabeculae is formed from the neural crest.

The only contribution of the neural crest to the formation of cranial ganglia is probably to the general visceral portions of VII, IX, and X. This conclusion is substantiated by the fact that when the crest cells are removed from the branchial and hyoid regions, there is a distinct lack of general visceral fibers, while the gustatory, lateralis, and general cutaneous fibers are normal.

SUMMARY

1. Above the optic vesicle in early stages of Amblystoma there is an elongated ophthalmic placode which gives off cells to the formation of the ophthalmic ganglion. When the ectoderm including this placode is removed as early as stage 23, the ophthalmicus profundus V nerve and ganglion are absent.

2. Near the anteroventral border of the supra-orbital lateral-line primordium is a small gasserian placode of brief duration which can be followed through stages 28 and 30. When a large area of ectoderm is removed from the region posterodorsal to the eye, deficiencies of the gasserian ganglion are produced.

3. Lying close to the anterior border of the auditory placode is a prominent placode elongated in a direction toward the dorsal extremity of the hyomandibular cleft. It can be located as early as stage 25. When ectoderm in this region is removed, even

before the appearance of the placode, the supra-orbital line of sense organs is absent as well as a large part of the VII lateral-line ganglion. The small remaining VII lateral-line ganglion in such cases gives rise to lateralis fibers, which innervate the group of sense organs on the lower jaw, and also slender fibers to the infra-orbital group.

4. The supra-orbital primordium of lateral-line sense organs arises from the anterior extremity of the VII lateral-line ganglion placode.

5. The supra- and infra-orbital and hyomandibular primordia of lateral-line sense organs have separate seats of origin.

6. The ventral hyomandibular and mandibular groups of lateral-line sense organs also appear to have separate seats of origin.

7. The epibranchial placodes of VII, IX, and X give off cells which become incorporated in the visceral ganglia, and when these placodes are removed from the ectoderm in early stages (23–26) no special visceral ganglia nor gustatory fibers can be found.

8. The complete removal of ectoderm in the region of IX and X which includes all the primordia of the lateral-line system is accompanied by a complete absence of lateral-line ganglia. When only partially removed, small lateral-line ganglia are produced.

9. Large areas of ectoderm removed from the region of IX and X also show an absence of cutaneous fibers as well as visceral sensory fibers.

10. The lateralis and special visceral ganglia are derived entirely from placodes, and the general cutaneous, for the most part if not entirely, is also derived from placodes.

11. The neural crest cells arise from the dorsal portion of the neural tube at the points of the fusion of the neural folds. They can be distinguished as early as the closure of the folds, and from this region they can be followed by their difference in pigmentation from the surrounding tissue and by the presence of small yolk granules in their cytoplasm as they descend upon the mesoderm of the visceral arches around which they wrap themselves and

later become arranged on the median surfaces of the arches where they form cartilaginous tissue.

12. The wandering mass of 'mesectoderm' is of neural-crest origin and in no region is it augmented by a contribution from cells of the lateral ectoderm.

13. The removal of neural crest in the branchial and hyoid regions is accompanied by smaller external gills and marked deficiencies in the branchial and hyoid cartilages. The hyohyals, ceratohyals, ceratobranchials, epibranchials, and first basibranchial are formed from the wandering neural crest, while the second basibranchial is formed from mesoderm near the anterior wall of the pericardial chamber. In the ganglionic regions the neural crest appears to form only the general visceral components.

14. The removal of neural crest in the trigeminal region shows no complete absence, but deficiencies in the size of the quadrate and mandibular cartilages. However, there is no doubt that these cartilages are formed from crest cells. The regeneration of the crest cells always occurred in the operation of these specimens because they were necessarily confined to very early stages.

15. The neural crest in the trigeminal region which migrates over the anterior border of the optic vesicles apparently gives rise to the anterior portion of the trabeculae.

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IS THERE A TRANSFORMATION OF SEX IN FROGS?

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This paper is a reply to the recent article of Dr. Emil Witschi which appeared in a late issue of the Naturalist (Vol. LV, No. 641). Witschi is quite convinced that the problem of sex development and differentiation in frogs has been settled, and that nothing further remains to be said. However, the writer feels that instead of being solved, the time has come for a revision of the entire question of sex development in Anurans, and that the subject is ripe for a reinterpretation upon a more rational basis than that accorded to it heretofore.

The first portion of the paper will be devoted to a brief exposition of the writer's interpretation of sex in frog larvæ based upon data obtained from a study of the bullfrog. The second part of the paper is a reply to certain questions raised by Dr. Witschi.

In larval males of the bullfrog two gonads are formed, just as there are two kidneys formed, a pro-testis or embryonic sex gland destined to degenerate and disappear in ontogenetic development and a definite or functional testis which replaces it. The germinal elements of the pro-testis arise in the entoderm and migrate into the germ ridges early in embryonic life. The cells multiply rapidly and together with the mesodermal elements of the germ glands form paired ridges projecting into the cœlomic cavity. While the tadpole is very immature and has yet a year of larval life before metamorphosing, the

germ cells of the pro-testis undergo a precocious and abortive sexual cycle culminating in degeneration and resorption. Beautiful cysts of spermatocytes are formed, but the first maturation division rarely proceeds past the

FIG. 1. Transverse section pro-testis *R. catesbeiana* tadpole. Animal has a year of larval life remaining. *A*, Spermatogonia showing nuclear polymorphism due to incomplete fusion chromosomal vesicles; *B*, Final spermatogonia; *C*, Spermatocytes in leptotene stage; *D*, Amphitene and pachytene; *E*, Heterotypic mitosis; *F*, Secondary genital cavity; *G*, Anlage of sex cords of definitive gonad which develops as a core within pro-testis.

anaphase owing to fragmentation of the centrosome and consequent formation of polyasters (Fig. 1). Sometimes aberrant spermatids are formed by suppression of the first and second maturation divisions and growth of axial filaments from the centrosome. Practically all the germ cells of the pro-testis degenerate and disappear while in various stages of maturation—some undergo an oviform type of degeneration, *i.e.*, hypertrophy enormously and take on the superficial characters of oocytes. The oviform type of degeneration, however, is more characteristic of the short larval-lived frogs than of *R. catesbeiana*, for in many animals these large cells appear rarely and in others not at all and this is an important point to keep in mind. This type of degeneration will be discussed in detail in a later paper; suffice to say it gives no clue to the sex of a cell. (See Plates 1 and 2.)

Some cells of the pro-testis fail to take part in the abortive sexual cycle persisting through the phase of maturation and degenerate as spermatogonia. These elements migrate into the sex cords (Fig. 1, *g*) which have formed meantime, and form a core of germinal tissue extending through the center of the pro-testis. This core of tissue plus the sex cords is the anlage of the definitive testis and is quite distinct from the pro-testis, the cells of which are maturating and degenerating, whereas the cells of the forming functional gonad remain as primitive spermatogonia. The definitive testis by rapid growth completely supplants the pro-testis which usually disappears some time before metamorphosis. The functional gonad is generally fully formed at metamorphosis when the larvæ are two years of age. Some tadpoles, but not all, develop ripe spermatozoa in the gonad at metamorphosis due to a second sexual cycle of the germ cells of the definitive gonad. (Swingle, '21, *Jour. Exp. Zool.*, Vol. 32.)

In the frogs with short larval-life the same succession of gonads occurs, but in these forms the developmental processes are greatly accelerated and the pro-testis ma-

turation cycle is cut short by the cells early becoming senescent and undergoing oviform degeneration *i.e.*, hypertrophy to such an extent as to superficially resemble oocytes. This oviform degeneration occurs to an even more marked degree in the progonad of the toad which has a still shorter larval-life, *e.g.*, in Bidder's organ. In male anurans the entire pro-testis or larval gonad is the homologue of the male organ of Bidder in *Bufo*.

The pro-testis of the short larval-lived frogs has been misinterpreted as an ovary owing to the oviform-type of degeneration characteristic of many of its senescent cells, and hence tadpoles are said to develop first as females, fifty per cent. later transforming into males. The normal embryological process by which the definitive testis develops as a central axis through the degenerating pro-testis or larval Bidder's organ, has been described by Witschi as the transformation of female tadpoles into males. In *R. catesbeiana,* where the larval life is prolonged over two years, the true nature of the pro-testis is revealed, for relatively few of the cells are of the oviform type and all transition stages between such cells and normal spermatocytes occur. The evidence presented by this material will be published in due time, and is too clean-cut to admit of any doubt that the entire larval gonad of male anurans is simply an embryonic male sex gland rudiment and not a temporary ovary. Witschi's Fig. 6 (this journal, Vol. LV), which he supposes is an ovary transforming into a testis is simply a transition stage in the development of the definitive testis, and degeneration of the pro-testis or Bidder's organ in a short larval-lived frog. Compare his Fig. 6 with Fig. 1 of this paper and note how the true male character of the cells of the pro-testis comes out in *Rana catesbeiana* tadpoles.

When the facts are considered it is evident that the transitory gonadic rudiment of male frog larvæ is an organ of Bidder which degenerates and is replaced by the definitive gonad. Any one who has studied the oviform-like

cells of the so-called sexually intermediate tadpoles and compared them with the cells of Bidder's organ in male toads, is at once struck by the remarkable similarity in their origin, development, structure and fate in the two groups. They are identical. The crux of the problem is the nature of Bidder's organ in male Bufonidæ and of the oviform-like cells of the pro-testis. The advocates of sex transformation have assumed that such cells are undoubtedly female, but no proof has ever been advanced that they are. Their ultimate fate is the same as that of the first year spermatocytes in the bullfrog tadpole — degeneration (see Plates 1 and 2). The sex-transformationists have been misled by the idea that everything superficially resembling an oocyte is necessarily such, or that any cell in tadpoles and first-year animals undergoing the early growth stages, leptotene, pachytene, etc., is to be regarded as female. These are fallacious criteria. Enormously hypertrophied oocyte-like cells which have passed through the early growth stages and entered the "germinal vesicle" period so characteristic of oocytes, occur as normal features of the male sexual cycle of certain animals, *e.g.,* myriapods (Figs. 5–8). These animals were at first regarded as hermaphrodites by Blackman (1905, *Bull. Mus. Comp. Zool.,* Harvard, Vol. XLVIII, no. 1) who found upon examination, however, that these "oocytes" were in reality spermatocytes of giant proportions, and developed into spermatozoa. The writer has examined some of Professor Blackman's material and the oocyte-like character of the male sex-cells is remarkable. In the material examined these cells practically fill the gonads. Firket, 1920, working on the chick embryo, describes and figures spermatocytes undergoing oviform degeneration, *i.e.,* enlarging to such an extent as to resemble oocytes. There are many other cases reported in the literature. How does Witschi know that the transitory oocyte-like cells he describes in the future male tadpoles or so-called hermaphrodites, are female cells and not senescent organ of Bidder cells occurring

in the course of the abortive and degenerate sexual cycle of an embryonic pro-testis?

The work of Witschi on the problem of sex in anurans can be summarized thus: He has described in great detail and with admirable exactness the process of development of the pro-testis or Bidder's organ in the short larval-lived frogs, its degeneration and final replacement by the definitive gonad. This process he calls transformation of females into males. The experimental investigations of Witschi upon sex transformation by environmental influences consists of this: By means of such agents as heat or cold, etc., he has simply modified the normal course of development of the pro-testis — Bidder's organ, thereby accelerating or delaying the development of the definitive testis. The experimental results show that it is possible to modify the developmental rate of the embryonic testis. Similar experiments carried out with regard to other larval structures would unquestionably give similar developmental modification. Cold hinders metamorphosis and all the normal structural changes metamorphosis implies. All of these environmental influences are interferences with the normal cycle of the gonads, by which the development of the definitive gonad out of the pro-testis is accelerated, retarded, or possibly prevented entirely. The following quotation from Witschi '14, page 10, is significant in this connection:

> Bei seinen Untersuchungen war es Hertwig aufgefallen, das unter dem Einfluss verschiedener Aussenbedingungen sich nicht nur die Geschlechtsziffern, sondern oft auch in ganz auffälliger Weise der Rhythmus, in Welchem die Keimdrüsen und manche andere Organe sich anlegen und entwickeln.

It is probable, judging from certain experiments reported, that the degree of development attained by the larval gonadic rudiment, its position in relation to the definitive gonads, its period of persistence, non-formation in some forms, and such like, may vary in different frog species and is determined by heritable factors. For example, in *Bufo*, the structure persists throughout life in

males, disappears after two years in females, and is anterior to the functional gonads. In frogs it forms the outer husk of the germ gland enclosing the centrally developing functional testis and may or may not show the oviform type of degeneration, *e.g., R. catesbeiana.*

If sex is so labile in tadpoles and young frogs, and females so readily transform into males under environmental stimuli, why is it that such sex reversals do not occur in adult frogs *after the degeneration of the protestis* and the formation of the definitive testis has occurred? All investigators are agreed that the sex ratio of adult frogs of all species reported is approximately 50–50. If environment (ever changing in the same locality, and never the same in different regions), plays such an important sex transforming rôle, why do male tadpoles never transform into females — all investigators agree that they do not. Why do only fifty per cent. of the so-called larval females transform into males if they were not zygotic males from the beginning, and why do not all female frog larvæ transform into males instead of only fifty per cent. if such transformation is possible? Appeal cannot be made to Professor Hertwig's well-known late fertilization experiments because in these experiments the influence of the over-ripeness of the egg upon the zygotic conditions determining sex are unknown. Hormones! To date there is no positive evidence that such secretions have ever actually changed a female germ cell into a functional male germ-cell.

Cases of hermaphroditism in adult frogs are thought by some to furnish evidence of a sex transformation in frogs. However, true hermaphroditism in adult frogs is as rare a phenomenon as it is in mammals when we consider the few recorded cases, and the enormous number of frogs annually dissected the world over. Crew ('21), *Journal of Genetics,* Vol. II, no. 2, has summarized the recorded cases of abnormal sexual organs in frogs and states that there are forty cases. To this number should be added a recent case described in the bullfrog, making

forty-one. Among these forty-one cases, there are but twenty-seven true hermaphrodites. Crew's cases, twenty-one to thirty-three, inclusive, are not hermaphrodites, nor is case thirty-eight, as none of the animals possess ovotestes and some are entirely without gonads. True hermaphroditism in frogs is a permanent and pathological condition, probably due to a mix-up in the genetic constitution of the individual, and is not to be confused with the present problem which has to do with a normal but transitory embryological process.

Much has been written about the marked "sex potencies" of various portions of the gonads in so-called sexually intermediate frogs, *i.e.*, females transforming into males. It is claimed that the outer rind of the gonad exerts a profound female sex influence, while the inner portion exerts a purely male influence. Germ-cells remaining in the outer husk (the main portion of the larval gonad by the way) of the gland are female, those migrating into the central part among the sex cords become male. All such speculations are based upon misinterpretations. The outer portion or husk of the larval male gonad is simply the pro-testis, the cells of which are undergoing a precocious maturation cycle just as they do in the organ of Bidder in *Bufo*, the inner portion or sex cord region is where the definitive gonad begins development and as it spreads and grows the embryonic male gonad degenerates and disappears. *It is in the region of most marked "female" tendencies that the writer finds in the bullfrog entire cysts of unmistakable spermatocytes, and occasional spermatids* (Fig. 1, *e*). In other words, the pro-testis — what Witschi regards as an ovary — can in the bullfrog, where its development is greatly prolonged, give rise to practically mature male sex products. Recently, the writer made an observation of considerable interest. In the degenerating Bidder's organ (pro-testis) of a two-year-old male larva in which formation of the definitive testis had been delayed until metamorphosis and in which the oviform type of degeneration

was the most marked of any animal yet observed, several cysts of unmistakable spermatocytes and spermatids were observed. They arose from the maturating cells of what Witschi regards as the female part of the gonad — in reality the pro-testis, and were of the cell type characteristic of the adult frog. This observation shows two things clearly: (1) *That the direct descendants of the male primordial germ cells (pro-testis elements) can produce practically mature germ cells;* (2) *that the spermatocytes of the structure regarded by the writer as a pro-testis are really male cells, and that the structure in so-called sexually intermediate frogs and tadpoles is in no sense to be regarded as female in character.*

Another point is of interest here — the writer has never observed direct testicular development in *R. catesbeiana,* though it probably occurs in some strains; the indirect method alone has been found, *e.g.,* first a pro-testis is formed which is later supplanted by the definitive gonad. In the bullfrog, which has the longest larval life of any anuran, the pro-testis persists longer than in other forms, sometimes two years before giving place to the definitive gonad. What the writer calls a pro-testis of so-called sexually intermediate tadpoles is according to Witschi a transitory ovary. If this is true why is it that despite its persistence for such a long time, relatively few oocyte-like cells are found in *R. catesbeiana* and in many individuals none, throughout a two-year period, but instead the structure produces spermatocytes and sometimes spermatids? Why is it, if this structure is an ovary in the so-called females that later transform into males, that the shorter the larval life of male anurans, the more the pro-testis in its structure and behavior resembles the Bidder's organ characteristic of male toads, due to rapid oviform degeneration of its cells; the longer the larval life, *e.g., Rana catesbeiana,* the more the germinal elements undergo a normal sexual cycle characteristic of male cells? The answer is, because in forms with extraordinary prolonged larval lives the

true nature of the embryonic male gonad has sufficient time to manifest itself before being supplanted by the definitive gland.

We come now to a discussion of the nature of Bidder's organ in *Bufo*, for this is the classical example of oviform degeneration of racially senescent germ cells. Heretofore, this embryonic sex gland rudiment has been regarded as characteristic of toads, but such is not the case. In frogs the pro-testis or larval gonad is a Bidder's organ, destined to be replaced by the definitive male gonad developing within; in male toad larvæ on the other hand, the functional gonad arises behind the pro-testis or Bidder's organ, consequently this structure persists as a degenerate gonadic rudiment attached to the functional gland.

According to the writer's view, Bidder's organ in *Bufo* is simply a vestigial larval gonad persisting throughout life and has the same sex as the definitive gonad behind it—male in males, female in females. It is just as though the pro-nephros of tadpoles persisted as a nonfunctional and degenerate rudiment at the end of the mesonephros. That many such larval and embryonic rudiments do persist through adult life in various animals is a commonplace of embryology, and their persistence in one species and total disappearance in another related one, is also well known. Bidder's organ in *Bufo* then, is a persisting, in frogs a transitory, embryonic sex gland rudiment, a relic of a phylogenetically earlier sexual condition. The functional gonads are more recently acquired structures (like the larval mesonephros) superimposed upon the older degenerate glands. Briefly stated, the evidence for the view that Bidder's organ is homologous to the pro-testis of frogs and that it is not a rudimentary ovary except in female animals is as follows:

1. The cells of Bidder's organ in *Bufo* are unquestionably germ cells. The gland appears very early in embryonic life (two weeks after hatching) and its cells far

outstrip in development the cells of the definitive gonads located posteriorly.

2. The cells of Bidder's organ extremely early in development undergo a precocious and abortive maturation cycle and become senescent and degenerate oocyte-like structures when the germinal elements of the functional gonads have barely started to multiply to form the definitive glands. This occurs in individuals of both sexes.

3. The larval maturation cycle such as occurs in the bullfrog, and in other anurans, throughout the entire larval gonad is confined to Bidder's organ in *Bufo,* and the changes occurring in this structure do not affect the normal developmental cycle of the definitive germ glands behind.

4. The so-called transformation of female animals into males, claimed by Witschi and others to be the normal course of development in frogs, does not occur in toads. Why? Because in *Bufo,* the definitive gonads are from the beginning located posterior to Bidder's organ, and it is not necessary in order that they may develop that this structure degenerate and disappear as is the case in frogs where the definitive testis starts development as a core within the pro-testis or Bidder's organ, necessitating its complete destruction.

5. Few have ever claimed that sex in toads is labile and easily reversed by environmental influences. Why? Because the sex of the definitive gonads is definitely fixed and clear cut at an early stage of life. The separation of Bidder's organ and the gonads has precluded the possibility of confusing the pro-gonad and the definitive gonad.

6. Bidder's organ is merely a persisting embryonic gonad whose cells have undergone oviform degeneration. It is not a rudimentary ovary except in female animals. This is indicated by its presence in both sexes in toads; its presence in Spengel's case of true hermaphroditism; by the fact that neither in male or female of toads do

its cells develop into true functional eggs; and by its degenerate structure from its inception in both sexes.

In a recent paper (*Zoologischer Anzeiger,* Dec., 1921) Harms describes marked hypertrophy of Bidder's organ following testis removal. He considers that castration of males causes Bidder's organ to develop into an ovary. However, it should be noted that such operated animals with hypertrophied Bidder's organ (ovary according to Harms) retain all their male secondary sex characters, and their normal mating instincts and that these male characters and instincts undergo a normal cyclical development in such induced "females." When Harms removed both testes and Bidder's organ the somatic sex characters and instincts failed to develop, showing clearly that Bidder's organ in male toads acts like a testis in maintaining the secondary sexual characters. This is excellent evidence for the writer's view that in male toads Bidder's organ is simply a persisting embryonic male sex gland rudiment and not an ovary. If it is an ovary why should it develop and maintain the secondary sex characters of the male in absence of the testis?

7. Recent investigators have inclined to the view that this structure is a hermaphrodite gland, *i.e.,* in male toads a rudimentary ovary, in females a rudimentary testis. If this is true then the admission is made that large, senescent, oocyte-like germ cells are not necessarily female cells — the crucial point for which the writer is contending.

8. Bidder's organ in *Bufo* corresponds to the larval gonad of frogs which in these forms disappears in the male and is replaced by the definitive testis. In the case of female anurans so far as the writer is aware no one has carried out a thorough investigation of the germ cycle from larval to fully adult life to see whether or not such a degeneration occurs in the female line. In mammals and birds such degeneration of the female embryonic line of germ-cells is quite well established as the work of Winiwarter, Firket and others shows.

The writer is of the opinion that it is only by adopting the view advanced here regarding the homologous nature of the larval male gonad of frogs, and Bidder's organ in *Bufo*, that the problem of sex differentiation in anurans can be placed upon a rational basis. The theory accords with the embryological facts, covers the experimental finding of Witschi and others, accords with our own cytological data in the bullfrog, accords with the embryonic sexual conditions of other vertebrates, *i.e.*, the degeneration of the embryonic line of germ cells in birds and mammals, and lastly furnishes an explanation of Bidder's organ in *Bufo*.

The key to the puzzle of sex development in frogs is simply this: every cell that superficially resembles an oocyte is not necessarily a female cell especially when occurring in an otherwise male individual, and that the larval male gonad of anurans is an organ of Bidder — a rudimentary embryonic sex gland with the same sex as the definitive gonad arising out of it. Misinterpretation of oviform hypertrophy and degeneration of racially senescent sex cells has rendered chaotic the problem of sex differentiation in anurans (see Plates 1 and 2).

Witschi regards the development of certain somatic sex characters such as the Müllerian ducts as very positive evidence for his theory of sex transformation. He says:

> In males which show a typical development of the testicles, no Müllerian ducts of any significance are formed. On the other hand, such animals as first develop ovaries and later undergo the transformation of sex, also show regular oviducts; and these continue to grow just up to the time when the transformation of sex begins. This parallelism in the behavior of the Müllerian ducts and the gonads furnishes definite proof that the "eggs" and "ovocytes," described by the writer, are in fact really eggs and ovocytes and that the transformation of sex is a well-established fact. After the transformation of sex, when the ovocytes have disappeared, the Müllerian ducts begin to shrink but they do not disappear completely, etc., etc.

The following data shows that in reality such so-called parallelism in the behavior of the Müllerian ducts and

the gonads does not exist and that evidence based on such parallelism is worthless.

In the normal males of adult *Rana pipiens* the Müllerian ducts are remarkable for their size and degree of development. They arise as cellular cords in the peritoneum at the time of metamorphosis and only acquire full development long after transformation when they come to resemble to a striking degree the oviducts of females (Fig. 2). In the larva of *R. pipiens* the so-called

FIG. 2. Urogenital apparatus of adult *Rana pipiens* showing the normal condition of the Müllerian ducts (*md*) in males of this species.

transformation of females into males (degeneration of the pro-testis and formation of the definitive testis) occurs very early in larval life, before the Müllerian ducts appear, and in this species the ducts undergo practically their entire development after the definitive testis has formed. In other words, while subjected to the influence of the fully formed testis and its ripening sex products the ducts undergo the most marked development known in the males of any anuran species. Moreover, in *Rana catesbeiana,* where if we accept Witschi's interpretation of femaleness, the so-called transformation of female in-

dividuals into males is a prolonged process requiring two years, and where the future male larvæ are subjected to the so-called female influence during the entire period, the Müllerian duct does not appear. At metamorphosis when the definitive testes are fully formed and spermatozoa are beginning to appear the cellular cords representing the vestigial Müllerian ducts of the male form but do not develop. If Witschi's interpretation were correct, one would certainly expect to find marked development and hypertrophy of the Müllerian ducts in *R. catesbeiana* because of their being so long exposed to female influence. As a matter of fact, these structures in the male bullfrog are less developed than in other forms.

The same criticism applies to the so-called developmental correlation of the Müllerian duct with the gonad of the same side in cases of lateral hermaphroditism. What Witschi terms lateral hermaphrodites are nothing more than larvæ or young frogs which show the definitive testis developing out of the pro-testis (larval male Bidder's organ) faster on one side than on the other. (See Witschi, Am. Nat., page 533.) In the end such animals develop into definite males with testes symmetrically formed. True lateral hermaphroditism in adult frogs is an exceedingly rare phenomenon. In the writer's material it is rare to find both definitive testes developing out of the pro-testes at the same rate, one gland may be the finished gonad, the other the pro-testis undergoing degeneration. Such larvæ are in no sense to be regarded as lateral hermaphrodites. There is no developmental correlation of the Müllerian ducts with the gonad of the same side in *R. catesbeiana* and *R. pipiens*, because there are no ducts formed until after the definitive testes are formed. Regarding the other somatic sex characters such as seminal vesicles and thumb cushions, it should be pointed out that the thumb pad in *R. catesbeiana* is not formed until after metamorphosis when

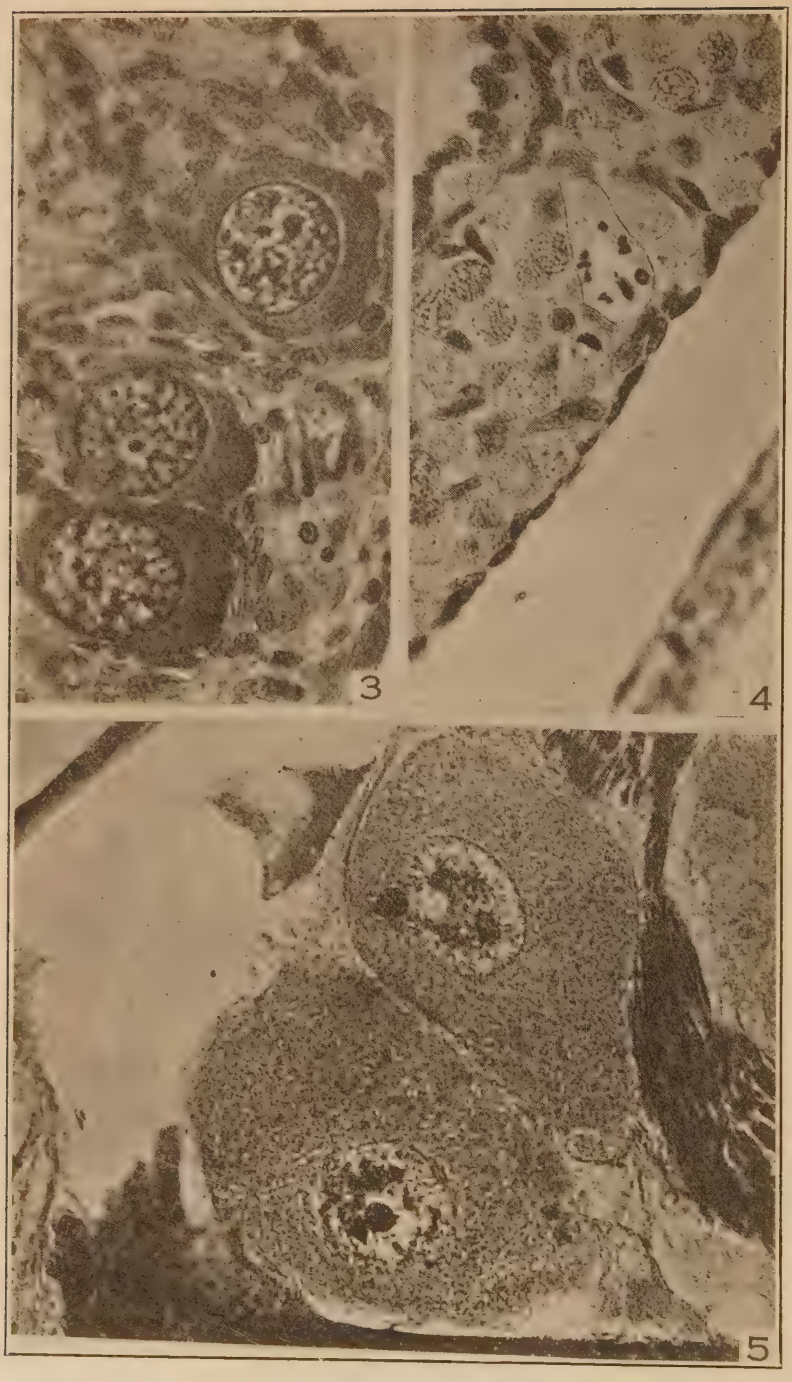

PLATE I

FIG. 3. So-called oocytes occurring in the degenerating pro-testis of larval bullfrogs. These cells according to the writer's view are merely hypertrophied spermatocytes that have undergone oviform degeneration.

FIG. 4. Section of pro-testis male larva before onset of degeneration. At *X* is spermatocyte in prophase. The black bodies are ring tetrads.

FIG. 5. The giant spermatocytes of *Scolopendra Heros* (Chilopoda). These cells form functional spermatozoa and make up the greater part of the testes. Note the "germinal vesicle" condition of the nucleus.

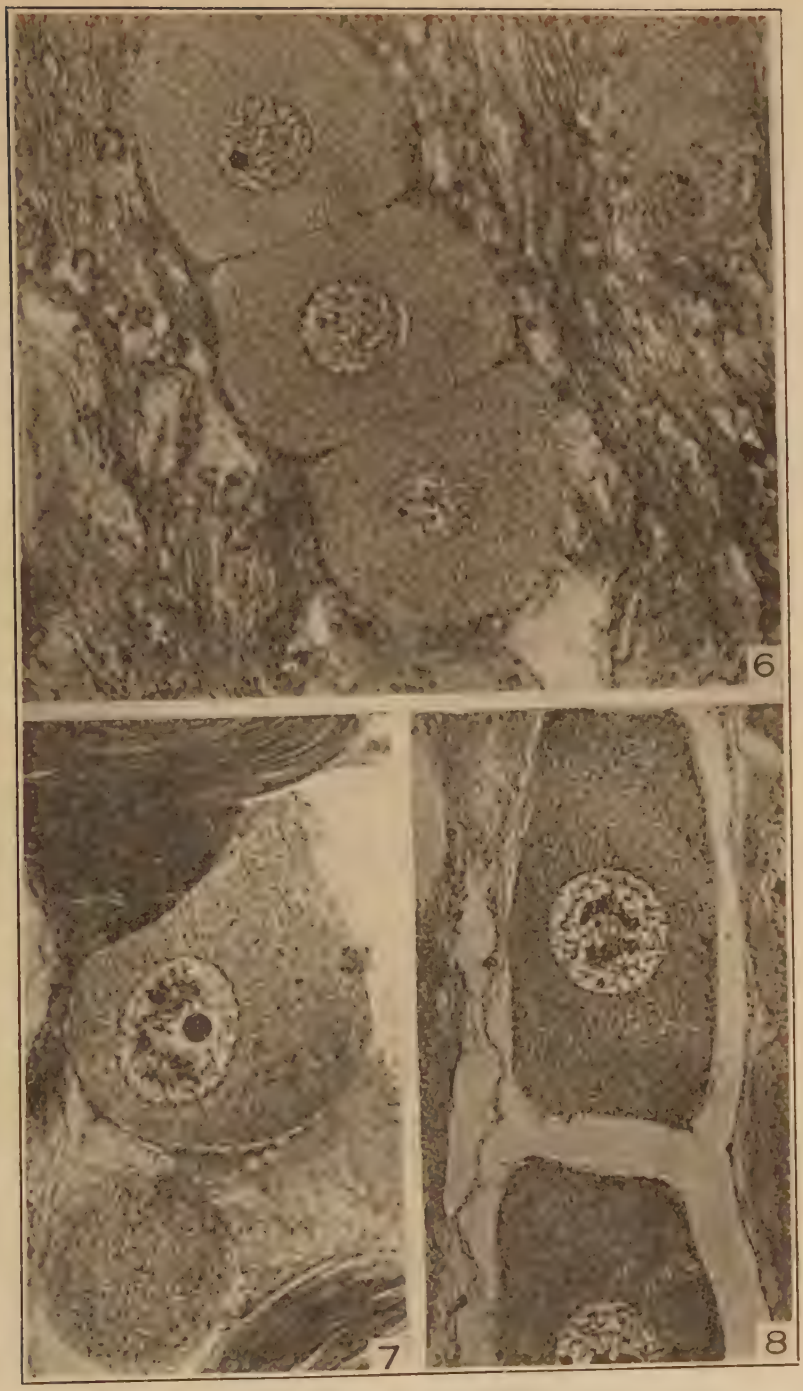

PLATE II

FIG. 6. Spermatocytes of *Scolcpendra.*

FIGS 7 and 8. Spermatocytes of *Lithobius* (Chilopoda). The resemblance to oocytes in the germinal vesicle stage is remarkable. Sections of the testes look like ovaries.

All photographs on Plates I and II made at a magnification of 500 diameters. No reduction. Figures 5–8 are from Professor Blackman's material.

the fully formed testes are present, and the seminal vesicles are absent or rudimentary in the males of many frog species, and exceedingly well developed in others.

In the few cases reported of true lateral hermaphroditism in adult frogs there is *not always* a developmental correlation of the Müllerian ducts with the gonad of the same side. Crew ('21), (*Journal of Genetics,* Vol. II, no. 2) has summarized the known cases of sexual abnormality in amphibians — see Figs. 7, 8, 9, 12, 14, and 16 of this paper, also the report of cases 21, 22, 23, 24, and 39. These are exceptions to any rule of developmental correlation. In several cases, Figs. 25 and 31, the ducts are quite as well developed in total absence of ovarian tissue as when such is present in large amounts, this, of course, being the normal condition in *Rana pipiens.* Crew also gives a list of frog cases reported where both gonads were entirely missing and yet the Müllerian ducts were well developed in such individuals.

Because of these facts it is fair to conclude that the appeal to the somatic sex characters completely fails as proof of the transformation of female frogs into males.

In closing, it should be pointed out that Witschi has made but one original investigation of sex in anurans (Witschi '14, no. 1). His later papers on the subject contain no new observations or experiments but are purely speculative endeavors to interpret his early work in accordance with Mendelism ('14, no. 2), later ('20, no. 3) in accordance with internal secretions.

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ZOOLOGY.—*The possibility of control of* Heterodera radicicola *and other plant-injurious nemas by means of predatory nemas, especially by* Mononchus papillatus *Bastian.*[1]

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I. INTRODUCTION

The investigations, the results of which are described in this paper, were carried on from December, 1921 to the end of May, 1922, in the Osborn Zoological Laboratory of Yale University, in collaboration with the U. S. Department of Agriculture. The work was outlined by Dr. N. A. Cobb of the Bureau of Plant Industry. As the investigations were of a novel nature, numerous methods for the rearing of nemas had to be worked out. A large quantity of soil-material was collected and washed by a combination of the sieve method described in an earlier paper by Cobb (7) and the well known gravity-method. The daily control of the cultures, transferring the predatory nemas to fresh conditions, keeping records of the victims and adding a fresh supply of food requires much time and patience. *Heterodera radicicola* material was kindly sent to us by the Connecticut Agricultural Station and from the Plant Introduction Garden of the Department of Agriculture, Brooksville, Florida. We wish here to express our appreciation for this assistance. We feel indebted to the authorities of Yale University, particularly the Osborn Zoological Department, for their permission to carry on our work and for their cordial cooperation.

II. THE PROBLEM

Numerous methods of control of plant injurious nemas have been described in the past. Although some of these methods are very useful, yet the fact remains that today the damage done by nema-pests is enormous and is still increasing.

In recent years the study of free-living nemas has been greatly increased, and as a result the problems connected with nema-pests

[1] From the Osborn Zoological Laboratory, Yale University, New Haven, Conn., in collaboration with the U. S. Department of Agriculture. Received September 9, 1922.

have acquired a new aspect. It was shown by Cobb (4,5,6), Menzel (13) and other investigators that there are in the soil certain species, and even whole genera, of nemas, which in some cases are, and in others may be, predatory and doubtless at least occasionally, are feeding on other kinds of nemas, and even species known as nema-pests. Cobb recorded a series of such observations (4) and suggested first the possibility of using these predatory nemas as a means of decreasing the number of plant injurious nemas in the soil.

In a more recent paper Dr. Menzel (13) compiled all the recorded facts and observations on food and feeding habits from the literature on free-living nemas. He performed also some experiments with a species of *Mononchus*, corroborating the observations of Cobb. He observed that *Mononchus papillatus*, brought together with *Tylenchus sp.*, *Plectus auriculatus*, *Tripyla media* and *Anguillula aceti*, attacked these forms, and killed them either by sucking out their vitals, or by swallowing them whole.

Cobb therefore advocated an investigation of the relationship between predatory *Mononchus* species and other soil-inhabiting and plant-infesting nemas, especially *Heterodera*. If possible the investigations should show to what extent the above mentioned facts regarding the feeding habits of some mononchs are a true expression of the life habits of these animals. If they prove to be a true expression, methods for the propagating and rearing of *Mononchus* should be studied with the view to applying the results for practical purposes in agriculture, especially for fighting the root-knot nema, *Heterodera radicicola*.

The first thing for us to find out was the life history, food, and feeding habits of the predatory mononchs. We chose for our investigations *Mononchus papillatus* Bastian, a species which appeared to be best fitted, first because it seemed one of the easiest to obtain, being among the commonest of mononchs, and second because many observations have been made on the voracity of this form.

Recent investigators (1–3, 8–12 and 14–18) have reared free-living nemas, but as far as we know, mostly forms which feed on decaying matter, and therefore in most cases easily reared in a small amount of suitable medium on slides or in watchglasses. Berliner and Busch and also Byars were the first who used agar as a culture medium for true soil nemas, although the nemas they experimented on were plant-parasites, namely: *Heterodera schachtii* and *Heterodera radicicola*. They planted seeds of oats, etc. on agar and then nematized it with eggs and larvae of *Heterodera*.

There have been seen no published statements made concerning the culture of predatory soil nemas. Menzel seems to have kept *Mononchus papillatus* specimens for only a short time in a small amount of water, simply in order to perform his experiments on their feeding habits. Therefore, it was first necessary to invent simple and practical methods for rearing mononchs.

III. METHODS OF CULTURE OF MONONCHUS PAPILLATUS BASTIAN

The greatest difficulty in rearing minute soil organisms, especially soil nemas, is to devise a culture medium permitting at any time the study and inspection of the reared animals and offering as far as possible natural conditions. Soil, however, even in small quantities, is not transparent. If then, the organism to be cultured is to be continuously observed, ordinary soil cannot be used as a culture medium.

A. Culture in a drop of water on a concave slide.—The first attempt to rear the mononchs was in a small drop of water on a concave slide. *Mononchus papillatus* specimens at different stages of growth were isolated in water-drop cultures, and *Heterodera* larvae, different species of *Rhabditis* or *Anguillula aceti*, added as food. By this method it was possible to keep mononchs alive for from one to three weeks, if the water was changed daily. In cultures of this sort the mononchs seemed to remain in very good condition for several days; they were easy to observe and study, and it was not difficult to get a record of the number of nemas eaten or destroyed by them. However, after a short time had elapsed, all animals reared in pure water cultures became sluggish and finally died.

B. Culture on concave slides with soil and water.—After a number of failures with the foregoing method, we tried to get more natural conditions by adding small quantities of soil to the drop of water on the concave slide. It was seen immediately that the nemas under such conditions grew better, and could be kept alive for a much longer time. After some experience, we were able to rear the mononchs by this method from the first larval stage up to the adult stage and even to the point of producing eggs. The following points should be kept in view.

1. In order to render the observations comparatively easy, only a small quantity of soil should be used. A larger amount of soil furnishes a much better medium for the nemas, but the investigator will have difficulty in finding and controlling them. By trials, any investigator can find the best amount of soil.

2. The nature of the soil is not of great importance. Our experi-

ments showed that sandy soil as well as other kinds may be used.

3. It should be seen to that the soil be free from decaying matter.

4. If possible the water, and even the soil, should be changed daily. *Mononchus papillatus* seems to be very susceptible to any pollution, especially bacterial (*B. subtilis* typ.).

It seems very astonishing at first that the simple addition of a small amount of soil should be of such great importance to the growth and life of these nemas. It was found that soil was absolutely essential for the keeping alive of newly hatched larvae and for rearing them.

C. The agar-culture method.—In 1914 Berliner and Busch described a method for rearing *Heterodera schachtii* Schmidt on agar plates. Agar was carefully washed, sterilized and poured into petri dishes. Sterilized seeds of oats, turnips, beets and different varieties of vetch and clover were planted on the agar and eggs or larvae of *Heterodera schachtii* added. This seemed to be a very simple and useful method also for our purpose, it being only necessary to add the mononchs to get a complete food cycle,—*Heterodera* feeding on the roots of the growing plant and *Mononchus* feeding on *Heterodera*. Byars in 1914 also described a similar method for rearing *Heterodera radicicola*. Had good results been obtained, it would have shown very beautifully the relationship between these two forms of soil nemas; but difficulties soon arose. In the first place *Heterodera radicicola*, the form used for infecting the roots, did not attack them (oats, cucumbers) and up to the present time our experiments along this line have not been satisfactory. The mononchs were already feeding on the *Heterodera* and seemed to thrive in the agar, moving constantly around, even laying a number of eggs. So we added more food for them, consisting of *Rhabditis pellio*, *Rh. elegans*, *Anguillula aceti*, and *Heterodera radicicola*. By this time the bacterial infection of the plate grew so heavy that the mononchs died. Young larvae already hatched from the eggs also died, even in freshly prepared and non-infected agar. Thus it seems to us that the agar method may be used only for larvae a week or more old, and adults, and then the agar should be changed every eight to fourteen days.

A further inconvenience consisted in the size of the plate, since on even the smallest plates it was quite difficult to find the nemas and eggs again, because of the rapid wandering of the former through the agar and the minuteness of the latter.

The second method, that of using concave slides with a small quantity of soil in a drop of water, seemed best fitted for our purpose,

especially in the matter of keeping records of the rate of growth and the number of animals destroyed.

D. The tube-culture method.—Desiring to control the results of our slide cultures, we started, after several other experiments, with cultures in a larger amount of soil in small glass tubes, which were placed in holes bored in a small block of wood, as shown in Fig. 1. A small

Fig. 1. Wooden block and glass-tubes with soil contents illustrating the tube-culture method of rearing mononchs.

amount of sterilized soil was placed in each of these tubes, moisture added, as well as the mononch to be reared, and a daily supply of *Rhabditis*, *Anguillula* or *Heterodera* as food. The tubes were kept in a moist chamber. If a large amount of soil was used it was not necessary to renovate the tubes for several weeks, but if a smaller amount was used, a weekly change was required. The chief aim was to secure conditions for the mononchs as near as possible to those found in the soil of fields.

Under these conditions it was possible to rear *Mononchus papillatus* in a shorter time than on concave slides. A larger number of eggs was also produced. If a sufficient amount of food was added, numerous mononchs could be reared together in the same tube, without their attacking each other.

The best method for inspecting a tube was to pour the contents into one or more large watchglasses and then dilute with a large quantity of water. If this was not done, eggs and larval forms easily escaped notice.

E. Remarks on flower-pot and field experiments.—Finally a series of host-plant experiments were also performed. Flower pots and soil were sterilized in an autoclave, and sterilized seeds of oats, tomato and cucumber, planted. The seeds were sterilized according to the pre-soak method, a one-tenth per cent solution of commercial formalin being used. After the first roots were formed and the growth began, the plants were infected with a certain number of *Heterodera radicicola* and also *Mononchus papillatus* added at the same time. Unfortunately

we worked with too small a supply of animals, and could not get noticeable results within the short time available. The roots were attacked by *Heterodera* but the effect of the *Mononchus* was not determined. With further experiments along this line, it should be quite possible to observe results from the external appearance of the host plants.

IV. THE LIFE-HISTORY OF MONONCHUS PAPILLATUS BASTIAN

Comparatively little is accurately known about the life-history of free-living nematodes. About the only forms that have been studied are those living on decaying matter, such as species of *Rhabditis*, *Cephalobus*, *Plectus* and *Diplogaster*. Most of these genera have a very rapid development and are in this way somewhat adapted to the ephemeras of dead and decaying bodies that serve as food. From a study of these forms it would be impossible to generalize and state that all soil or free-living nematodes have a rapid development and short life. The results we obtained in rearing *Mononchus papillatus* showed that the life of this form may be of considerable length, and the same thing may be true of many of the other soil nematodes.

In order to judge the value of *M. papillatus* for fighting other nematodes it is necessary to have a complete knowledge of its life history, the time required to reach the adult stage, the number of eggs produced and the length of life after the cessation of egg production.

Nematodes live in the soil as members of an association of living forms, among which there exist interrelationships. It is important to know what effect *Mononchus* as a predatory form may have on the associated nematode population of the soil, and especially its influence on the diminution of forms causing root-knot and similar diseases; if the monoch has a high rate of propagation, and lives for a long time, it has naturally a more rapid and beneficial effect.

A. The hermaphroditism of Mononchus papillatus.—Hermaphroditism in numerous species of the genus *Mononchus* was first observed by Cobb. Males have been described in very few species, and seem to be extremely rare in most of them. A gradation exists in this genus similar to that found in the genera *Rhabditis* and *Diplogaster*, where we find some species in which there is an equal number of males and females, some in which from ten per cent to forty per cent or more are males, some species in which not even one per cent are to be found and still others in which males seem never to occur.

Conditions similar to these are found in the genus *Mononchus:* the Antarctic *M. gerlachei* seems to produce an equal number of males

and females,—males are rare in *M. obtusus* and in most of the species they have not yet been found.

Hermaphroditism in *Mononchus papillatus* is of some importance in our problem, as every individual is able to reproduce, not being dependent upon the presence of the other sex for propagation. This may prove of great value in using this species as a means of fighting injurious nemas.

B. The egg and the embryonic evolution.—The spermatozoa in *M. papillatus* are very minute, the ovary producing them early, and later on the eggs. Fertilization then takes place. The ovary of the nema is syngonic, and the animal a protandric syngone. (See Cobb 5.) We could not determine the number of sperms produced but it is probably rather restricted, as we observed several cases of old females that formed eggs (at times depositing them also), that were probably not fertilized, as they never developed.

There were never more than two eggs in one uterus at a time, and usually two or three to the individual were observed. It was rather a rare occurrence for the animal to have four eggs, two in each uterus. The eggs were elongated, and appeared smooth shelled in the uterus, but when deposited the surface seemed covered with protuberances, having the appearance of short ridges, the whole surface having a somewhat lace-like effect. (See Fig. 2.)

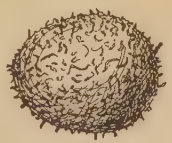

Fig. 2. Egg of *Mononchus papillatus* showing the protuberances on the egg shell.

If the mononchs lived in an approximately normal condition, an average of two or three eggs was deposited daily. Our slide cultures showed a production of two or less, but the tube cultures gave higher results, and it may be that under natural conditions in the soil the egg production is still higher.

In the slide cultures, the maximum number of eggs deposited by one female was twenty-eight in twelve days, the animal then ceasing to produce; but in tubes our records showed eighteen eggs produced in six days, and forty-one larvae hatched from eggs produced by one female in twenty days were also found. The exact number of eggs produced may have exceeded this, as even with the most careful inspection, eggs and larvae may escape notice. Experiments along this line are still being carried on and higher figures may be obtained.

The number of eggs laid by one *M. papillatus* seems very small when compared with that of some other free-living species, as, for instance, species of *Rhabditis*, in which within a few days eight hundred or more

eggs are produced. The maximum number obtained from one *Mononchus* was forty-one, the minimum number, twenty eggs.

The eggs required from six to seven days before hatching. This also is rather a long period compared with *Rhabditis*, *Diplogaster* and some other known forms. The mononch eggs are unicellular when deposited; the first division takes place about twenty-four hours later.

C. The development of the larva of Mononchus papillatus.—The newly hatched larva is very active but not hardy, and the presence of soil seems very essential to its life. The body is quite transparent. As far as our experiments show, during the first two or three days the larvae attacked no nemas; but on the fourth day, and even on the third day, the predatory instinct was developed and they began to feed on other nemas. As soon as this occurred the intestine began to darken and in a short time became opaque.

The time of larval development up to the period of sexual maturity was somewhat varied. Slide cultures showed the average time for larval development (from the end of embryonic life in the egg shell to sexual maturity) to be from six to seven weeks. But tube cultures showed the minimum time to be only four and one-half weeks. The temperature of these cultures was the same, and the difference in results must be due to the more natural environment found in the tubes. Therefore we are inclined to adopt the minimum time as the time required for development, and to judge from this the influence of this species upon the nematode population of the soil.

Unfortunately, up to the present, we have not been able to get exact data as to the time and number of the moultings. We observed three moults, two in the earlier stages of growth and one just before arrival at sexual maturity. In a study of the life history of one specimen we observed a moult at eighteen days and another at forty-four days after hatching, the latter being the moult just before sexual maturity. Judging from the size of the nema at which other moultings were observed, there must be a moult between eighteen and forty-four days. There may be a moult soon after hatching, but we never observed it. The skin is shed as a whole. There were no observations of the act of moulting, but, judging from the cast, the animal leaves the skin through an opening in the ventral side near the cardia. The mouth-capsule and wall of the rectum were found attached to the cast; the lining of the oesophagus was not observed and may be cast off separately. The animal became rather sluggish and inactive just before each moult.

D. The adult and senile Mononchus papillatus.—Soon after moulting the last time, the mononch began to produce eggs. At this age, the nema was extremely voracious. The number of eggs produced seemed to be in direct proportion to the number of destroyed and devoured nemas.

The maximum duration of egg production observed was twenty days, but further experiments may probably show a longer period.

The period after the cessation of egg production was called the senile stage. It seemed very remarkable that this senile stage was of such long duration as compared with the length of the other periods. In one case the senile period lasted ten weeks, while the combined length of the other periods was only eight weeks. In many hermaphroditic nematodes there are long senile stages. The senior author observed in cultures of the hermaphroditic *Rhabditis elegans* Maupas, the length of the different life periods to be as follows:

April 20—newly hatched larva,—(larval stage lasted two days or less).

April 24—first production of eggs,—(egg production stage lasted two to three days).

April 26—production of eggs stopped,—(senile stage lasted thirteen days).

May 9—death.

Of a life period of eighteen to nineteen days, thirteen were of the senile stage, that is, the period after the last fertilized egg was laid. The senile stage was therefore two or three times longer than the other life periods taken together. The protandric syngonism (Cobb 5) of these forms is probably the cause of this. If spermatozoa are essential and only a certain number of spermatozoa are produced, then when this supply is exhausted, if the gonads are unable to produce more sperms, all subsequent ova will fail of further development, and senility begins.

The same thing seems to happen in *Mononchus papillatus*. The comparatively enormous length of the senile stage may be the result of the inability of the syngone to produce sperm cells a second time. The senile period of ten weeks was observed on a slide culture, and under more favorable conditions it may last very much longer.

In judging the relationship between *Mononchus papillatus* and other soil nematodes the above facts are of some importance, as the amount of food required during the senile stage was very small compared with that devoured during the egg-producing period. Consequently all

senile *M. papillatus* have a decreased efficiency in combating nematode pests.

V. LENGTH OF LIFE OF M. PAPILLATUS

Eighteen weeks was the maximum length of life observed for *M. papillatus* in a slide culture; the tube culture method showed the life period under more normal conditions to be very much longer. Compared with the length of life of other nemas, even excluding forms of *Rhabditis*, *Diplogaster*, *Plectus* spec., etc. that live on decaying matter, this period seems very long, but it may not be exceptional for soil nematodes. At the Zoological Station of Cette (France) the senior author kept adult marine nematodes alive for a comparatively long time, as for example an adult species of *Thoracostoma* for over thirty days. We may therefore conclude that free living nematodes have a much longer life period than is usually supposed.

To a certain degree, this fact may compensate for the restricted number of eggs of *M. papillatus* when considering the effect of this species on the nema population of the soil and the whole community of life therein, as, it means an increase of the efficiency of this species as a destructive factor.

VI. FOOD AND FEEDING-HABITS OF M. PAPILLATUS

In working out our problem, a study of the food and feeding habits was of the greatest interest. From statements made by the investigators already mentioned, it was known that *M. papillatus* fed on other nematodes. It was necessary to discover whether the carnivorous habit lasted throughout its life or for only a certain period. To obtain these facts was of great importance as they determined largely whether *M. papillatus* can become a really efficient agent in combating nematode pests. Our experiments along this line confirmed fully what Cobb in several papers stated to be true. In our cultures, *M. papillatus* lived exclusively on other nemas, also appearing at times to devour small soil particles. The consumption of soil grains seemed to be of chief importance in the first larval stage of the animal, and we observed likewise in the intestine of adults soil particles of unknown origin. The important influence of the presence of soil on our cultures seemed also to confirm these observations.

The larval *M. papillatus*, just hatched from the egg, was very active. Its intestine at first was transparent; the cells of the thin wall at this stage were easily seen, and their nuclei as well. But in a suitable medium, a few days were sufficient to transform this colorless intestine

into a brownish, or even apparently black organ. To this end the presence of soil was absolutely necessary. In water, but with plenty of food (other small larval nematodes) the mononch larvae remained transparent and died in a short time. We were not able to determine exactly what kind of food the newly hatched and very young larvae ate. They moved through the soil, sucking here and there on soil fragments, and particles of an organic nature were probably consumed. But the mononch larvae soon began to feed on other nematodes. We observed three-day old larvae feeding on *Rhabditis* larvae, and their voracity increased with their age.

Our records showed that the number of nematodes killed daily in-

Fig. 3. Photograph of the dead bodies of *Rhabditis elegans* Maupas as they appeared after their slaughter by mononchs. The picture shows well the common method of destruction, that of piercing the cuticle and sucking the contents of the body in the manner of a weasel.

creased as the mononch developed, beginning with one or two destroyed during the third or fourth day of larval life, and attaining a maximal number of sixty-five. In one case as many as eighty-three *Heterodera radicicola* were killed (either swallowed wholly or partly sucked out) by one mononch in one day. The largest amount of food was consumed at the time the maximal number of eggs was produced, and then it decreased during the senile stage of life. During a life time of about twelve weeks one animal killed 1332 nematodes. We are certain

that this number may be very much larger under natural conditions. It was also observed that during the moulting periods fewer nematodes were killed and for a period of one or two days none at all, with a decided increase after the moult.

When in a resting position, the mononch lay on its side, and never on either its back or "stomach." The body was more or less curved ventrally,—the tail end often bent in the form of a spiral. At times the whole body was spirally curved. Agar was the best medium for the study of the movements. It was seen that locomotion was accomplished by bending in the dorso-ventral plane of the body, this plane being placed mostly in a horizontal position so that the dorsal and ventral sides were seen in profile. *Mononchus papillatus* changed continually from a moving to a resting state, moving backward as well as forward.

In the water-drop cultures with a small amount of soil, the mononchs were found only very exceptionally outside the soil area. There was a striking difference in the behavior of *Mononchus papillatus* and the *Rhabdites* added as food. The mononchs seemed to have a more highly developed stereotropism, as they remained well hidden in the soil, while the *Rhabdites* were more or less concentrated on the edge of the water drop outside the area occupied by the particles of soil.

The sense of touch seemed to be the only sense *M. papillatus* used in hunting about for food. It is known that in nematodes three different kinds of sense-organs may be developed.

1 *Organs for the sense of touch.*—These are no doubt among the chief sense organs of the group and show a high degree of perfection in stereotropic reactions and reflex movements.

2 *Chemical or chemico-physical sense organs,*—those known as lateral organs or "amphids." In *M. papillatus* the amphids are rather small and inconspicuous.

3 *Organs for the perception of light.*—These are found in many free-living nematodes, but are absent in mononchs as well as in most soil inhabiting forms.

Judging from the behavior of *M. papillatus*, the organs of touch were probably the only ones used while hunting for food. The nema moved through the medium, continually searching its surroundings by moving the head end in all possible directions. The head end contains the chief organs of touch, as well as those for chemical perception. After long observations, we came to the conclusion that *M. papillatus* was not able to find its prey at long distance, and the use

of the sense organs of touch seemed to be its chief method of locating food. We could therefore confirm statements made by Menzel about this matter.

As soon as the head end of a mononch came into contact with its prey, it grasped it tightly. By a sucking movement, probably of the oesophageal muscles, the head was fixed to the prey. The nematode caught by the mononch naturally made most violent efforts to get free, and often the mononch was shaken and dragged along. But it held on tightly and soon its tooth and other mouth parts began to work. By a sucking movement of the oesophagus and mouth cavity, the mononch became more firmly fixed to its prey, when the tooth located on the dorsal wall was protruded and produced an opening in the skin of the victim. Then a stronger (or further) sucking action was exerted by the mononch and the tooth came back to its normal position. The whole oesophagus came often into action and moved forward and backward with the intestine, the whole body going through the same movements; first the body fluid of the prey was sucked out, and as this happened its body became shrunken. Very often the mononch was satisfied with this, and set free its prey, which died shortly afterwards. Specimens of *Rhabditis elegans* killed in this way are pictured in Fig. 3. *M. papillatus* rarely devoured an entire animal when feeding on large forms, except when exceedingly hungry. No one part of the victim's body seemed to be preferable for attack, the prey being seized at random. Fig. 4 shows an *Anguillula aceti* attacked near the cardia, and

Fig. 4. Photograph of a mononch attacking an *Anguillula aceti* near the cardia. Photograph taken from an agar-plate.

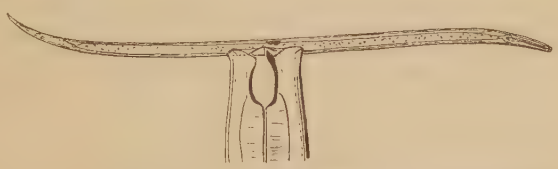

Fig. 5. Sketch of the head-end of a mononch attacking a larval *Rhabditis*. Notice the wide opened lips and the protruded tooth.

Fig. 5–9 in other situations. Larval mononchs were only able to attack and destroy the prey in the way described, but even a small mononch will attack prey of much larger size. Large mononchs often swallow smaller nemas whole; this will happen, if the prey is seized at one end of the body, the animal being then sometimes swallowed without being killed or even injured by the mouth organs of the mononch. In our slide cultures this phenomenon was twice observed. A large adult mononch swallowed a rather large larva of *Rhabditis elegans*. In this species of *Rhabditis* the larvae are very slender and more resistant than other species. The larva seemed to have passed through the mouth and oesophagus of the mononch without harm, probably in the way sketched in Fig. 10. We first noticed that the mononch, very active the day before, appeared nearly motionless. To our great astonishment we noticed the larva of the *Rhabditis* moving throughout the intestine as if searching everywhere for an exit. The larva moved in every possible direction, the mononch becoming less active all the time. We were very interested in knowing the outcome of this case. The digestive fluid in the intestine of the mononch did not seem to harm the larva. The same evening, the mononch did not react to outside stimuli and appeared quite motionless. During the night the *Rhabditis* found a way to freedom through the vulva opening, and in the morning the mononch was dead. The same thing happened shortly afterward with another very active mononch.

Fig. 6. Sketch of the head-end of a mononch seizing an adult *Rhabditis* by the neck. The skin of the prey is already opened, and the mononch is sucking the contents; notice that the dorsal tooth during this sucking action is in its normal position and not protruded. The prey was afterwards sucked out as shown in Fig. 9.

Fig. 7. Sketch of a *Rhabditis* seized near the middle of the body and partly sucked. See Fig. 8 for later stage.

From a scientific point of view these incidents are of interest, as they show that this species of *Rhabditis* is very resistant to mononch

digestive fluids, and they also throw light on the way the mononch swallows, and the action of the dorsal tooth of the mouth cavity. Incidents of the kind just described seem only possible if the tooth, during the act of swallowing, is protruded to such a degree that its point cannot harm the prey. Very probably, the latter was caught by the tail-end, and in the act of seizing, the tooth was protruded and the tail glided into the mouth cavity. In most cases the prey is seized at some other part of the body as Figs. 7–8 show. In this case not only the body fluid is sucked out, but also a large part of the intestine. Fig. 8 shows the prey after release, the skin remaining.

Fig. 8. The *Rhabditis* refered to in Fig. 7 as it was released after having been partly sucked. Notice that the skin of the emptied part remained.

The chief organ of the mononch functioning during the act of swallowing was undoubtedly the oesophagus. The contractions of its radial muscles were vigorous; they began at the mouth cavity and ran down the oesophagus so quickly that at first they seemed instantaneous. But more careful observations showed that each contraction first began around and behind the mouth and then ran back to the intestine. This contraction of the oesophageal radial muscles was accompanied by a shortening of the entire organ, and the whole intestine during the act of swallowing moved back and forth. As soon as the radial muscles contracted, the oesophagus apparently shortened and the forward movement of the cardiac region was easily seen. When contraction ceased, the oesophagus assumed its normal shape and the cardiac region and intestine moved backwards.

Fig. 9. Sketch of a *Rhabditis* sucked out completely at one end, notice the remaining skin.

The mouth cavity did not serve as a place for macerating food, but more or less as a suction capsule. Together with the lips and the attached muscles, it may seize and retain the prey, but our knowledge of the whole apparatus was not sufficient to completely understand its function. The dorsal tooth was certainly protruded as long as the whole cavity was used in retaining the prey.

Some of the digestive fluids are probably formed and secreted by small glandular cells among the radial muscles of the oesophagus. These glands were first described by Cobb. Their secretions may not act in the oesophagus itself, as the food did not stay there. Digestion began and ended in the intestine. The oesophageal glands were probably compressed every time the animal swallowed by the shortening and pressure of the radial muscles, and the digestive fluid thus entered the intestine with the food.

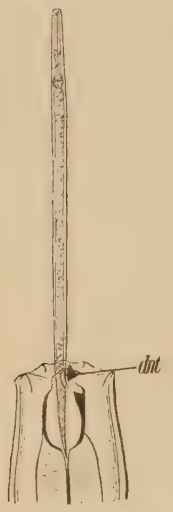

Fig. 10. Sketch of the supposed manner of swallowing the *Rhabditis* larva which was found living and moving in the intestine of a mononch, killing it afterwards by breaking through the intestine and body wall. *dnt.* —tooth as it is supposed to have acted during the attack on the larva.

VII. MONONCHUS PAPILLATUS AS A FACTOR IN FIGHTING NEMATODE PESTS

The foregoing experimental results show conclusively the predatory nature of *Mononchus papillatus*. From our experiments, and from observations made by other investigators, all kinds of nematode species, and Rotifers, Naididae (small Oligochaetes), etc. are taken as food. *Heterodera radicicola* is acceptable in large numbers, and we were able to rear *M. papillatus* by feeding this form of plant-injurious nema exclusively. The same thing happens in the soil and there is no doubt but that mononchs live there in about the same way as in our cultures. Undoubtedly this predatory nema when present kills *Heterodera* and other injurious forms in soil planted with crops. It is therefore extremely useful and its propagation should be encouraged. Under some conditions this form perhaps completely controls *Heterodera* and similar plant-injurious species. Why should this not become the case in our infested fields? Further investigations should be started along these lines.

Once in the roots, *Heterodera* and other such root-parasites are probably protected against the predatory mononchs, but when moving freely in the soil during larval life, they may be destroyed in large numbers and we are convinced that under favorable conditions *Mononchus* is able partially, perhaps completely to control some of these destructive forms. In order to advance any further along this line, it is absolutely necessary to have a knowledge of the nematode population of different kinds of soil, of the relationships that exist

between these forms and other members of the community of living forms that exist there, and of their relationships to other nematode species.

VIII. THE SOIL, ITS LIFE AND THE NUMBER OF NEMATODES, ESPECIALLY OF PREDATORY FORMS THEREIN

Our knowledge of life in the soil, especially of microscopic life there, is still very restricted. We know very little about the relationships that exist between the living components of the soil, and often we do not even know these components. Certainly soil fertility does not depend entirely upon its chemical, colloidal and physical nature, but also and probably largely, upon the animal and plant life therein. The importance of soil bacteria is already known and there exists a close relationship between these bacteria and the nematode population, as a large number of nemas are bacteria consumers. Investigations carried on throughout more recent years have definitely proved that the soil contains an enormous number of nematodes, and that, next to bacteria, they form probably the largest constituent element in subterranean life. Numerous new species have been described and all investigators agree that there is an enormous number still undescribed. It will require the collaboration of many research workers to describe all these forms. A nema species may furnish millions of individuals to every acre of our fields. No exact records regarding the number have been published so far. Dr. Cobb kindly placed at our disposal some of his tables from field surveys. The results of the surveys shown below are of great interest.

	Minimum number of nematodes per acre, top six inches (15.2 cm.)
From Missouri corn field	648,000,000
From North Carolina field	242,400,000
From New Jersey field	129,600,000
From Rhode Island field	610,800,000
From New Hampshire field	99,600,000
From Minnesota field	121,200,000
From Vermont field	580,000,000
From Kansas field	278,400,000

These tables give only the minimum numbers and are figures taken from a study of only the top six inches (15 cm.). A maximum penetration of some nema species has been observed to be as much as (7–8 m.) 25 feet. These unpublished observations were made by Dr. Cobb on citrus trees in California and on alfalfa roots in New South Wales.

Usually soil nematodes go as deep as the roots of plants can penetrate and therefore the depth figures recorded in publications are too small.

Until recently nemas have been almost completely disregarded by investigators of soil economics, and especially by research workers in soil fertility.

If we wish to know what part nematodes play in the economics of the soil, one of the first questions to be answered is what is the nature of their food. There have been no very comprehensive studies made of this question up to the present, and our knowledge is based mostly on only occasional observations. The results of these show that some forms such as *Rhabditis*, *Cephalobus*, etc. feed on decaying matter, that is on the contained bacteria or bacterial products; others, such as *Tylenchus*, *Heterodera*, *Aphelenchus*, etc. feed on plant tissues or fluids; others such as *Dorylaimus*, etc. feed probably on both plants and animals; and still others such as *Mononchus papillatus*, described in this paper, are apparently exclusively predatory. So that not only the problem of combating nematode pests in soil by the use of their natural enemies will be attacked by investigations along this line, but also the problem of soil fertility itself.

Today we know that soil nematodes play a very important role,

1. As consumers and destroyers of our crops;—often destroying whole fields, but much oftener decreasing their yield in a less perceptible degree.

2. As consumers of, and as important workers in the distribution of the bacterial flora of the soil. Their activity is certainly not restricted to denitrifying bacteria, but extends to nitrifying forms as well. A closer study may show relations of very great importance. They carry bacteria and fungus spores everywhere. Wounds on roots or other parts of plants may very often be infected by bacterial and fungus diseases carried by these nematodes. (See Metcalf 15.)

3. As having relationships with fungi upon which some species are known to feed. According to Zopf fungi on the other hand may use nematodes as prey.

4. As consumers of protozoa. It is known that certain fresh-water nemas feed specially on protozoa, sometimes apparently on a single species. There is a probability that some soil species may feed in a similar way, and further investigations may enable us to combat plant injurious protozoa by the use of these nemas.

5. As furnishing a control for plant-injurious nemas. This refers to the work of predatory nemas, such as *Mononchus papillatus*, whose

feeding habits have been described in this paper. There are other species of nemas with a similar significance. Here is a great field for study. Investigations should also be carried on to show more definitely the relations of these predatory forms to rotifers, oligochaetes and other soil animals.

6. As a factor in the humification of the soil, in transformation and removal of all kinds of organic matter, decaying bodies, etc.

7. As assisting in the aeration of the soil. We know how very important aeration of soil is for the growth of bacteria and all kinds of plants. Quite possibly these millions of nematodes living in the soil are highly useful aerators.

In order to solve the problem of the relation of soil nemas to soil fertility, we require a knowledge of the nema population in the soil itself and we also need to know:

1. The composition of the nema population, the species found and their numeric representation in all kinds of soil,—sandy, humus, swampy, dry-soil, hard clay, etc.

2. The horizontal and vertical distribution of nemas in all kinds of soil and the reasons for this.

3. The seasonal changes of the different species, the number of species and the number of individuals of each species.

4. The influence of habitat on the nematode population including physical, chemical and biological factors found in the habitat. The biological factors include kinds of crops and other forms of vegetation that may be present.

The working out of these problems will surely enable us to conclude why nema pests are absent in some cases, and why in apparently similar conditions they exist in such enormous numbers. A large field is thus opened up for investigations, the results of which will be of great importance to agricultural science.

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EXPERIMENTS ON THE METAMORPHOSIS OF NEOTENOUS AMPHIBIANS

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TWO TEXT FIGURES AND TWO PLATES (EIGHT FIGURES)

The discovery of the causal relationship existing between certain endocrine secretions and amphibian metamorphosis has served to stimulate anew the interest of investigators in the problem of neoteny and paedogenesis as presented by various species and genera of this vertebrate group.

The retention of the larval form either permanently or for periods far beyond the normal time required for metamorphosis is known as neoteny or neotenie (Kollmann, '82). This author, who has studied the problem carefully, distinguishes between partial neoteny, where the animal is simply retarded in metamorphosis beyond the normal time and passes the winter as a tadpole, and total neoteny, in which case the animal retains its gills and other larval characters becoming sexually mature in this condition.

Partial neoteny is quite common in such frog species as R. clamata and R. catesbeiana as there is a pronounced tendency of these forms to prolong the larval life an extra six or eight months beyond the usual one- and two-year period. Indeed, the larval life of Rana catesbeiana may extend to the period of sexual maturity in some tadpoles in so far as the possession of ripe spermatozoa is concerned, and this appears to be the explanation of the second larval sexual cycle described by the writer in a previous paper ('21).

The classical example of total neoteny is axolotl, the paedogenetic larva of Amblystoma tigrinum. The researches of Duméril ('65), Chauvain ('75) and others on the metamorphosis of axolotl have resulted in the more or less current belief that the

peculiar group of aquatic amphibians known as perennibranchiates (Necturus, Proteus, Typhlomolge, etc.) are permanent larval forms capable of reproducing the species. The group is supposed to represent a sort of retrograde evolution from an originally terrestrial life to permanent aquatic existence by suppression of metamorphosis. The evidence for this view is suggestive; briefly stated it is somewhat as follows:

1. It is generally conceded that the perennibranchiates do not form a natural group, but are to be regarded as a heterogeneous assembly; various genera are undoubtedly represented in the group. These animals probably became neotenic at a phylogenetically old stage and are hence the oldest and not the youngest members of the present-day urodeles.

2. Various anatomical features of the group, such as the pentadactyloid limb, presence of lungs, suppression of internal gills, and connection of the pelvic girdle with the vertebral column, point to a terrestrial existence somewhere in the history of the group.

3. Perhaps the most suggestive line of evidence for the view that the perennibranchiates are permanent larval forms is the occurrence of neoteny and paedogenesis as aberrations of development in semiterrestrial species of urodeles. For instance, it has long been known that the larvae of certain European salamanders fail to undergo metamorphosis and occasionally attain the size of 80 mm., whereas the normal size at transformation is 40 mm. Larvae of Triton have been reported 80 to 90 mm. long with functional gills and sexual organs fully developed. DeFilippi ('61) found in one locality in Lombardy sexually mature larvae. According to him, such gill breathing, sexually mature specimens occur constantly in a small lake in the province of Ossola in the Italian Alps. Many other cases have been reported, and the classical example of the axolotl is well known. It will be recalled that this creature was classified by systematists as a distinct species of perennibranchiata until Duméril described its metamorphosis into Amblystoma tigrinum.

4. Lastly, it has been repeatedly stated in the literature that one of the perennibranchiates, Typhlomolge, is hereditarily

lacking in the thyroid apparatus, which, if true, accounts for the suppressed metamorphosis. It will be recalled that it is possible to induce neoteny in anuran larvae by thyroid extirpation.

This evidence is suggestive, and taken in conjunction with what we know of the thyroid gland and its relation to metamorphosis suggests that the thyroid mechanism of forms such as Necturus, axolotl, and neotenous anuran larvae is defective and incapable of bringing about transformation.

EXPERIMENTS ON PERENNIBRANCHIATES (NECTURUS MACULATUS)

A group of adult necturus were obtained from the Ohio Valley and repeatedly injected with thyroid extract, and at the same time forcibly fed large quantities of the desiccated commercial preparation by means of a long glass pipette thrust down the throat. This procedure was repeated several times with negative results, despite the fact that the thyroid dosage was relatively enormous. The physiological activity of the thyroid preparation was tested by feeding small amounts to larvae of R. clamata, averaging 50 mm. total length, but without hind legs. These animals promptly showed marked indications of metamorphosis within eight days from the date of feeding (fig. 7). The iodine content of the desiccated tissue was given on the label of the bottle as 0.21 per cent by weight and the analysis made by the chemists of Parke, Davis & Company, Detroit. It is obvious that the thyroid preparation used in the experiment was not responsible for the negative results. One animal was injected twice intraperitoneally with 10 mg. of thyroxin iodine obtained from the laboratories of E. R. Squibb & Sons, and then forcibly fed large quantities of desiccated thyroid and anterior pituitary lobe substance. The physiological activity of the thyroxin iodine was tested by placing two young specimens of Amblystoma punctatum in a 1 to 50,000 solution, whereupon they metamorphosed within two weeks. None of these agents, singly or taken together, produced the slightest indications of metamorphosis, nor, I may add, appeared to harm the Necturus in any way. As a last resort, three thyroid glands of newly metamorphosed

Rana clamata frogs were engrafted subcutaneously in one animal. Four months later, no metamorphic changes had appeared. The experiment was abandoned, with the conviction firm in the writer's mind that, although adult Necturus may possibly be induced to metamorphose, thyroid tissue alone is not the agent that will accomplish the transformation. Axolotls readily respond to thyroid administration by metamorphosis according to Laufberger ('13) (cited by Adler, '16), Huxley ('20), and others.

Examination was made of the thyroids of untreated Necturus, but nothing unusual was observed, except that in some animals the glands are small and the vesicles more or less isolated from each other, while in other animals the glands may be large. The blood supply appeared normal in those individuals with compact glands. The thyroid of one animal consisted of but four to six extremely large follicles on either side, in others the gland was larger and comprised ten to sixteen large follicles, while in one animal the glands consisted of twenty-one or twenty-two follicles on each side. In the last-mentioned case the animal had been treated with thyroid and thyroxin iodine several weeks previous to examination.

In order to test the physiological activity, a series of heteroplastic thyroid transplantation experiments were made. In the first set of experiments one-half of the thyroid gland (from one side only) was engrafted intraperitoneally into immature Rana clamata tadpoles averaging 52 mm. total length, with hind-leg buds 2.7 mm. long, but undifferentiated. Within eight days the tadpoles which received the graft showed all the symptoms of hyperthyroidism. The animals were greatly emaciated, with protruding eyes; the hind legs increased in length from 2.7 mm. to 8 mm. with complete differentiation. In two animals the right fore leg had appeared and the tail atrophied to half its original length. At the end of the eighth day from the date of transplantation the larvae measured 26 mm. total length, whereas the week previous the same individuals averaged 52 mm. It was impossible to keep the tadpoles alive until they completely metamorphosed, so great was the acceleration of metabolism and metamorphic change. Death in most cases was apparently

due to respiratory difficulties. Several tadpoles were kept alive until tail resorption was nearly complete by placing them in shallow containers and passing a stream of compressed air through the water.

In a later series of experiments the thyroids were cut into small pieces and each part transplanted separately into immature tadpoles without hind limbs. One animal received three large colloid-filled vesicles dissected out of the gland; another received four follicles; the remainder received seven large follicles each. The grafts were made July 26th, and on July 30th, when examined, several engrafted individuals showed evidences of hyperthyroidism, such as emaciation and limb development. By August 2nd all of the animals showed a marked reaction to the graft; emaciation was very marked, the eyes protruded, and the legs had greatly increased in length. One animal had the right fore leg through the skin, and the remainder showed autolysis of the skin in the region where the fore limb later appears. The control animals remained unchanged (figs. 3 to 6). August 7th, when the experiment was discontinued, all engrafted animals were in advanced stages of metamorphosis. During the course of the experiment the animals were fed quantities of Spirogyra. They fed very little after the first three or four days, and not at all following the onset of marked metamorphic change.

This experiment shows clearly that the thyroid glands of adult Necturus are highly active metamorphosis-inducing agents. It is reasonable to assume that if they are capable of producing marked metamorphic changes when transplanted into immature anuran larvae within eight days, they are potentially competent of doing likewise in Necturus if this animal still retained any capacity to transform. It will be shown later that the relation of the thyroids to metamorphosis in Necturus is quite different from the situation existing in axolotls and neotenous anurans.

While writing this paper the writer came upon a statement by Uhlenhuth ('21) that Jensen ('14) had subjected Proteus to the action of thyroid substance, but did not get any demonstrable results. Uhlenhuth's comment upon this experiment is interesting. He says: "Many causes may have been responsible for this

failure (i.e., Jensen's), in particular the fact that the animals were too old when they were subjected to the thyroid feeding." And elsewhere the same author states "that nothing is known of the endocrine system of Proteus."

The writer ventures to predict that if the thyroid glands of an untreated Proteus are engrafted into anuran larvae, the latter will react similarly to those engrafted with Necturus glands. These experiments negative any such assumption as Uhlenhuth's ('21, page 201) that, "if the thyroid substance is capable of causing the development of the characters of a terrestrial amphibian, the administration of thyroid substance should cause the metamorphosis of Proteus anguineus." This writer apparently holds the view that any perennibranchiate will, if fed thyroid, metamorphose, and that retention of larval characters in these forms is due to absence or defect of the thyroid mechanism. The present experiments lend no support to any such hypothesis. In regard to the statement that the endocrine system of Proteus is unknown, it is interesting to note that Franz Leydig in 1853 (p. 62), describes the thyroids of this animal. According to his description, they apparently do not differ greatly from those of Necturus.

Uhlenhuth lays great emphasis upon the fact that one of the perennibranchiates, Typhlomolge rathbuni, is said to lack the thyroid gland, and states in effect that the reason for the retention of the larval characters is due to the thyroid absence. Perhaps this is true, but it strikes the writer as being rather odd that the retention of larval characters of this perennibranchiate should depend upon the absence of the thyroid function, and that such characters are retained in another form (Necturus) in spite of the presence of a most potent and active thyroid, in spite of feeding, injecting, and engrafting of thyroid substance.

There is another point of interest in connection with the discussion of Typhlomolge, and that is the apparent absence of thyroid glands in adults of this peculiar animal. Emerson ('05) studied the general anatomy of two specimens preserved in 4 per cent formalin, and merely mentions in the course of her discussions, "Sections of the head reveal the presence of a thymus

gland, but I do not find thyroids." This is the only mention made of the thyroids in her paper. Recently, through courtesy of Prof. H. H. Wilder, the writer had an opportunity of examining Miss Emerson's material, consisting of serial sections through the head of one animal. No trace of a thyroid was observed, but it should be stated that some of the epithelial structures had disappeared.

The writer has carefully examined three adult specimens of Typhlomolge and failed to find any trace of a thyroid. The entire lower-jaw region, back to and including the heart, was dissected under a high-power binocular microscope, and some tissue sectioned, but with negative results. However, the failure to find the glands does not necessarily mean that they were not present or had not been present at some period, because the animals had been preserved over fifteen years in alcohol and many of the epithelial structures had undergone disintegration during this interval.

If the thyroid mechanism of Typhlomolge is congenitally lacking, then this amphibian is the only vertebrate known in which the gland is normally absent. It has been stated in the literature that Typhlomolge is only the neotenic larva of Spelerpes, but it is well known that Spelerpes larvae possess thyroids. It seems probable that the Texas cave salamander has a thyroid, but that it develops as a diffuse aggregation of follicles, somewhat similar to the condition known to exist in teleosts. At any rate the question of the presence or absence of the gland deserves further investigation before it can be accepted as an established fact, because the history of vertebrate morphology is replete with descriptions of forms supposedly anomalous for the lack of certain structures, only to be later shown to possess them.

To sum up, it may be said that these experiments indicate that Necturus and probably other perennibranchiates have permanently lost their ability to metamorphose into terrestrial forms under the stimulus of thyroid administration alone: our experiments indicate that the thyroid apparatus of these animals is highly active and potent despite their larval characters.

These experiments, however, do not rule out the possibility of inducing the metamorphosis of perennibranchiates by other means than that of thyroid feeding or transplantation. The cause of the non-metamorphosis of these forms may be pluriglandular in origin, and a result of defective interrelation of various endocrine glands. It should be added that the writer fed one Necturus small quantities of desiccated ovarian, testicular, adrenal, and anterior pituitary lobe tissues, along with large quantities of thyroid extract, but without avail. It is probable that transplants of these various glands simultaneously would have had more effect than feeding the desiccated substances, in case the animal possessed the capacity to transform. Administration of endocrine secretions, no matter in what quantity given, can give positive results only when acting upon an appropriate hereditary substratum. The indications are that the hereditary factors concerned in the metamorphosis of Necturus have become so modified that the appropriate substratum is lacking, thus rendering the thyroid hormone powerless. It is obvious that hereditary conditions in the perennibranchiates are quite different from those in axolotl, in regard to metamorphosis, since the latter readily respond to thyroid feeding and the former do not.

EXPERIMENTS WITH AXOLOTL THYROIDS

Through the courtesy of Prof. Henry Laurens, of the Department of Physiology, the writer obtained a very large specimen of Axolotl mexicanum (neotenic larva of Amblystoma tigrinum) for thyroid transplantation work. The animal was a very large one, measuring 14.25 inches from snout to tail-tip. The exact age of the specimen is unknown, as it was obtained, along with several others, from Albuquerque, New Mexico. When first brought to the laboratory the animal was about 8 inches long, and hence presumably about two years of age at the time; it was kept under laboratory conditions for two more years, thus making four years the animal's approximate age when used by me. This specimen was the only one of the lot that failed to metamorphose within a few months following removal from its native habitat to New Haven.

The thyroid glands were compact and large—larger than the glands of newly metamorphosed R. clamata frogs—and made up of large and small follicles filled with colloid. The blood supply to the glands was rich, apparently much more so than is the case with larval anurans; only a superficial examination was made to test this point because of the lack of sufficient material.

The pituitary gland was also examined; the pars anterior seemed normal or possibly rather small when the relative sizes of all the lobes are considered.

The thyroids were dissected out and each gland cut into three pieces of approximately equal size. Each piece of tissue was then transplanted intraperitoneally into immature Rana clamata larvae averaging 51.5 mm. total length with hind-leg buds 2.2 mm. undifferentiated. A few hours following grafting one tadpole jumped out of the container and was found dead, leaving but five transplanted tadpoles. Eight days after transplanting the pieces of axolotl thyroid, the engrafted tadpoles showed all the characteristic features of hyperthyroidism, such as cessation of growth, marked acceleration of limb development, tail atrophy and resorption, and body emaciation.

August 20th, or twelve days after transplantation, four of the engrafted animals were found dead (figs. 8 to 10). The photographs show very well the marked tail atrophy and resorption and the fore-leg development. The early death of the animals was due to the great acceleration of metabolism and metamorphic change. Undoubtedly, smaller pieces of the axolotl thyroid would have had the same effect upon metamorphosis without the too destructive rise in katabolic activity. The amount of tail resorption can be judged by the fact that during the twelve days of the experiment the average total length of the tadpoles decreased from 51.5 mm. to 29.6 mm. The control animals remained unchanged.[1]

[1] Since this was written one hundred and nine large axolotls were obtained by Professor Harrison from Albuquerque, New Mexico, and given to me for experimentation. This experiment was repeated on a large scale with identical results. Mr. Carl Mason, of this laboratory, metamorphosed thirteen normal, thyroidless, and pituitaryless R. sylvatica tadpoles by transplanting pieces of the thyroid of a single 14-inch axolotl.

The pituitary gland of the axolotl was also engrafted into an immature larva of R. clamata, measuring 45 mm. total length, hind limbs 2.5 mm. long and differentiated into thigh and shank, but without toe points. All three lobes of the gland were transplanted together. The success of the graft was attested by the color change in the larva induced by the expansive action of the pars intermedia secretion upon the melanophore system.

Eighteen days after the graft was made the animal had not changed in any way either in regard to limb development or growth, so the experiment was abandoned. Examination of the implanted gland showed it to be mostly resorbed.

The negative result following grafting of the axolotl pituitary is in striking contrast to that obtained when the pituitary of a newly metamorphosed frog is transplanted into immature anuran larvae. However, it must be remembered that in the latter case we are dealing with a homoplastic graft and in the former with a heteroplastic one, and a single transplant at that. It is unfortunate that a larger amount of axolotl material was not available, for it is of importance to know whether or not the pituitary gland of the axolotl is active, and some idea of its potency can be obtained by testing its effect upon limb development of anurans. If the results are consistently negative, then it is probable that the gland is defective in so far as its relation to the thyroid mechanism is concerned.

The results obtained by grafting portions of axolotl thyroids, are clear-cut and admit of but one interpretation: namely, that the thyroid apparatus of this animal is highly active and potent in inducing marked metamorphic change when transplanted into immature anurans, but is apparently incapable of initiating metamorphosis when left unmolested in its normal place. This experiment seems to rule out the idea that the axolotl's thyroid secretion is defective. If the thyroid glands of a single axolotl when cut into six fragments are capable of initiating metamorphosis in five anuran larvae grafted with a single fragment (the sixth animal died) within ten to fourteen days, surely we may safely assume that the same glands, entire, contain enough of the active hormone to initiate metamorphosis in the single axolotl of which

they originally formed a part. The metamorphosis of an anuran larva involves much more fundamental transformation and reorganization of tissues and organs than the same process in axolotl.

Our experiment suggests four possible factors to account for this anomalous situation: 1) Possibly the blood supply taking the hormone away from the gland is defective and the thyroid consequently unable to release its secretion. 2) The thyroid is able to collect, store, and transform the incoming iodine taken from the food and water into the physiologically active hormone, but owing to defective nervous stimulation the gland is unable to release the secretion into the blood stream. 3) The secretion, though perfectly formed, is unable to escape from the gland, owing to some defective interrelation between the pituitary and thyroid, or possibly some other endocrine gland which supplies the necessary stimulus to the thyroid, thereby acting as the releasing factor. 4) The blood and tissues of neotenous forms may contain substances that neutralize or render impotent the metamorphosis-inducing agent of the thyroid hormone.

If it is true, however, that the thyroid hormone is unable to escape because of defective outlet through the blood stream, or because of defective interrelationship between various components of the endocrine system, how is it that axolotls usually promptly metamorphose when taken from their native habitat or when subjected to sudden environmental changes, such as a change from New Mexico to New Haven?[2] Furthermore, aside from the nervous system, there are few anatomical or physiological mechanisms which hold such power over other structures as to permit a gland like the thyroid to manufacture and store in large quantities a highly complex substance, but apparently prohibits its release. When we consider the thyroid glands of an axolotl filled to capacity with highly active secretion, as our experiments clearly show, yet apparently unable to release the

[2] Of the 109 axolotls received from Albuquerque, only those that were thyroidectomized failed to metamorphose spontaneously a few weeks after removal from their native habitat. Left unmolested in the New Mexican environment, the animals may remain permanent larvae and grow to a length of 14 inches.

hormone into the network of capillaries surrounding the gland in sufficient quantities to induce transformation, we are led to the conclusion that in the last analysis the crux of the problem is defective stimulation (perhaps inhibition) of the gland by that portion of the animal's nervous system responsible for the flow of secretion under normal conditions.

How else can one reduce to harmony the multiplicity of factors that have been invoked to explain axolotl metamorphosis, save by reducing them all to a common factor: i.e., agents which produce their effect by subjecting the organism to more or less violent changes of the environment thus acting as a constant nervous stimulant? For example, a few of the agents (aside from thyroid or iodine feeding)[3] that have served to initiate axolotl metamorphosis are: sudden changes in food supply, drying of swamps or pools in which the animals live, changes in the temperature of the water (Shufeldt, '85); forcing the animals to breathe air, insufficiently aerated water (Chauvin, '75, '77); administration of salicylic acid (Kaufman, '18); shifting of the animal from its normal habitat to other districts, such as from New Mexico to New Haven.

A glance at this list of factors indicates that their varied nature alone negatives the idea that any one of them can be the real causative factor in axolotl metamorphosis. However, all can be classed as shocks to the organism, and it may possibly be that such more or less constant excitation may bring about nervous stimulation to the thyroid sufficient to overcome the inhibiting influence and release the stored secretion, thus initiating metamorphosis. The nature of the inhibiting factor is of course the crux of the problem, and in the last analysis is probably of endocrine origin acting through the intermediation of the nervous

[3] Apropos of Uhlenhuth's claims that iodine has nothing to do with axolotl metamorphosis, the recent papers of Jensen and Hirschler are of interest. Jensen (Compt. Rend. Soc. de Biol., T. 85, 1921) metamorphosed axolotls by injections of iodocasein, iodoserumglobulin, and iodoserumalbumin. Also by feeding with an organic iodine compound—iodo-thyrosin. Hirschler (Arch. Entw. Mech., 1922) metamorphosed axolotls and anuran tadpoles by feeding elemental iodine in various forms. These investigators worked on strains of axolotls which do not spontaneously transform.

system. On theoretical grounds, the writer believes that electrical stimulation, thyroid puncture, and extirpation of the thyroid and reimplanting it into the same individual will metamorphose axolotl, but to date has been unable to obtain sufficient animals for experimentation along the lines indicated.

If such procedure should cause metamorphosis, then it is clear that the physiologically active hormone is not released from the thyroid in sufficient quantity to induce transformation. From the evidence at hand it seems to the writer that such is probably the case. Axolotls readily respond to thyroid feeding or to injections of iodothyrine by transforming, and the amount of thyroid substance required is not excessive. If the assumption were correct that the blood and tissues of this neotenic form contained substances which neutralized or rendered impotent the thyroid hormone, thus preventing metamorphosis, why should small amounts of thyroid substance, when fed, be able to produce an effect? The evidence obtained from thyroid transplantation experiments with neotenous anurans is interesting in this connection.

EXPERIMENTS WITH THE THYROIDS OF NEOTENOUS ANURANS

The larvae of the green frog, Rana clamata, have a larval period of approximately one year: i.e., 370 to 400 days from the date of egg deposition. The animals attain a length of about 65 mm. at metamorphosis, which occurs in late July and early August. However, it has been repeatedly observed by the writer that many larvae fail to transform at the usual time and remain an extra year as tadpoles. Such animals are typically neotenous forms and, as they continue growing throughout the larval period, they ultimately reach a size considerably in excess of that generally exhibited by the species at metamorphosis. Larvae measuring 75 to 90 mm. total length, with differentiated hind legs varying from 4 to 20 mm., have been captured in the months of November, December, and January from various pools in the vicinity of New Haven. The tendency of the species is to prolong rather than curtail the span of larval existence. Because of this fact, a series of thyroid-transplantation experi-

ments were carried out with these neotenous individuals, in the hope of determining the endocrine locus responsible for the failure to metamorphose at the proper time. The first procedure was to test the physiological activity of the thyroid apparatus of such animals by transplanting the glands into immature larvae of the same species with undifferentiated legs and noting the effect upon metamorphosis.

December 17, 1920, three immature larvae, averaging 33.5 mm. total length, without hind limbs, except undifferentiated epithelial buds, were engrafted with the thyroid glands of 80-mm. neotenous larvae with hind legs 11.6 mm. long. The engrafted forms were the smallest obtainable at this season of the year and not over six months of age. It is unfortunate that a larger number could not have been used, but the results are clean-cut, as will be seen later, when the experiment was rechecked with another group of animals in midsummer.

One animal was in advanced stages of metamorphosis on January 17th, just one month from date of grafting. The right fore leg was through the skin and the left fore leg appeared the day following (January 18th). For a week previous to the first appearance of the fore limbs the engrafted animal showed marked signs or hyperthyroidism, such as emaciation, protrusion of the eyes, slight tail atrophy, and autolysis of the skin over the region of the fore legs. The control larvae at this time averaged 34 to 38 mm. in length and showed no change in regard to leg development from the condition when the experiment was started. The other two larvae of the engrafted culture developed fore limbs January 22nd and typical frog mouth. All of the animals died before tail resorption was complete. Figure 1 is a drawing of an engrafted larva and its control. Figure 2 shows the neotenous type of tadpole from which the thyroid glands were taken. Generally the limb development is less marked than the drawing indicates. When the drawings were made the animals were nearly a year past their normal time of metamorphosis.

Briefly summarized, the experiment shows that transplantation of the thyroid glands of neotenous larvae 80 mm. total length, with differentiated hind limbs 11.6 mm., into immature tadpoles

33.5 mm., without limbs, brings about very marked metamorphic changes within thirty days, although the animals could not be reared to the stage of complete tail resorption.

It was observed that in the engrafted animals autolysis of the skin over the region of the fore limbs occurs independently of limb development as a distinct phenomenon of anuran metamorphosis. Years ago Braus ('06) described similar phenomena in developing tadpoles after extirpation of the limb bud. I mention it here because of the remarkable autolysis which is sometimes observed in transplanted larvae; the fore limbs may be small, whereas the skin area destroyed may be very large indeed compared with limb size. It should be remembered, however, that in anurans the fore and hind legs tend to keep pace with one another in development, only the fore legs are not visible because of the opercular covering.

The chief point of interest, however, is the odd fact that much greater metamorphic change follows transplantation of the thyroids of 80-mm. neotenous larvae with hind legs 11.6 mm. into immature larvae without limbs than occurs in control animals 80 mm. with legs 11.5 mm. In other words, the grafted glands wrought far greater changes in the same time interval (approximately one month) when transplanted into immature larvae than when left undisturbed in the mature forms.

This result, so curious and at variance with what one might expect, led to a repetition of the same experiment the following summer, from a different angle. Immature larvae averaging 40 mm. total length with hind leg buds 0.5 mm. were transplanted with the thyroids of mature though not neotenous tadpoles 68 mm. total length with hind legs 11.5 mm. The results were similar to those of the earlier experiments, though not so striking as one would expect, because the mature control animals (not neotenous) in this experiment were approaching metamorphosis at the end of the experiment, whereas in the previous experiment the neotenous controls showed no change, and in fact passed the winter in the laboratory as tadpoles.

The experiments indicate that the thyroids of extra-season, neotenous anuran larvae with hind limbs 11 mm. long are physio-

logically active and capable of inducing metamorphosis, provided their contained secretion could get into the blood stream. Like the thyroid mechanism of axolotl, the glands of these larvae seem to be rendered more or less functionless by an inhibiting factor which prevents secretion of the hormone into the circulation. Following transplantation, the inhibition apparently is overcome by the acquisition of a new blood and nerve supply

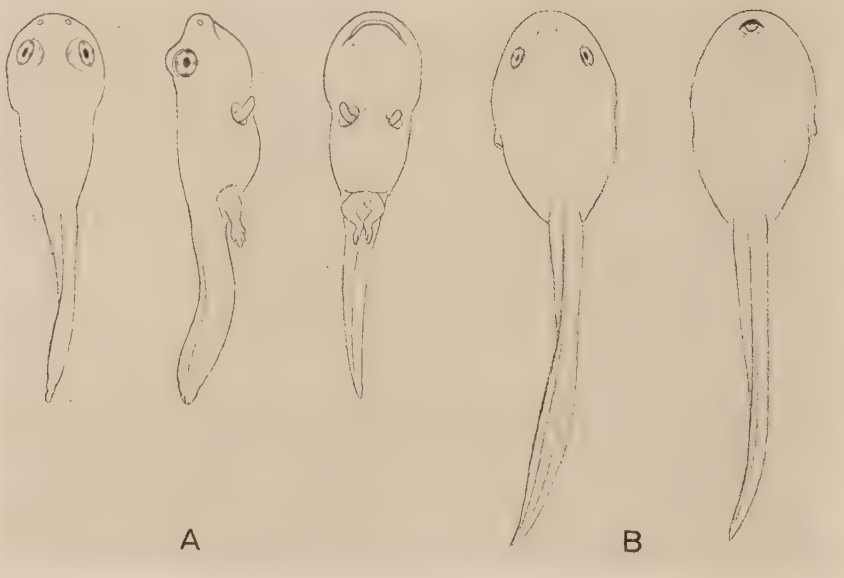

Fig. 1 Immature R. clamata larvae engrafted with the thyroids of extra large neotenous tadpoles with hind limbs 11.6 mm. long, B, immature control animals of A groups. × 2.

in the new environment, because the absorbed secretion induces metamorphosis.

A modification of the experiment just recorded was attempted; the thyroids of large neotenous larvae were engrafted intraperitoneally into other extra-season animals of the same size and developmental stage. The idea was, that since the glands of such individuals are physiologically active and capable of

inducing marked metamorphic changes when transplanted into immature animals, they would probably produce similar effects in neotenous larvae while undergoing resorption in the foreign

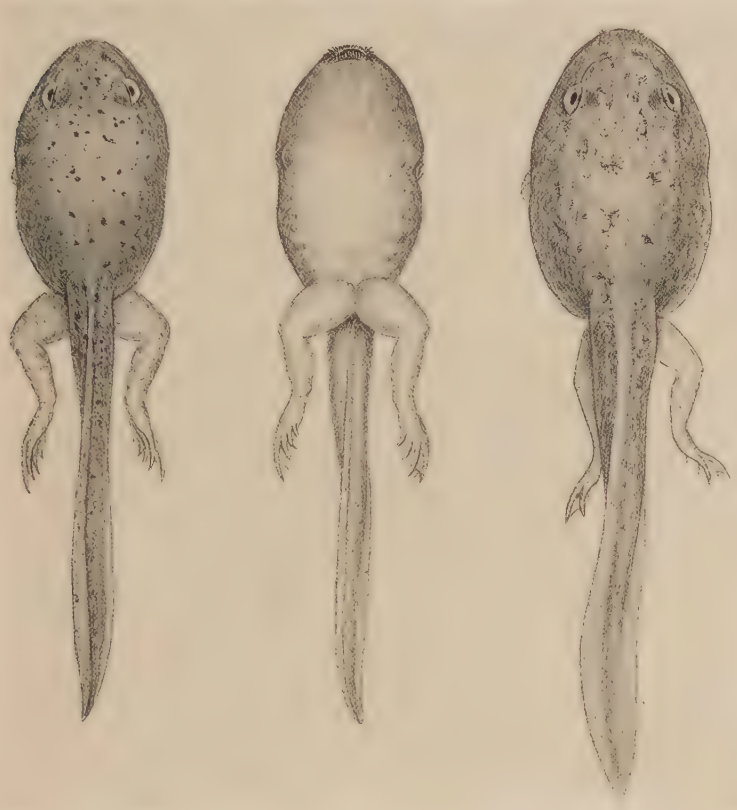

Fig. 2 Neotenous R. clamata tadpoles. Numbers of this type of larvae, measuring 75 to 90 mm. total length, with hind limbs 5 to 25 mm. long, pass an extra year beyond their usual period of metamorphosis in this stage. Drawing natural size.

environment because inhibiting factors could not prevent the release of the contained secretion. The experiment is briefly presented in the following tables:

TABLE 9

December 22, 1920

ENGRAFTED		ANIMALS FROM WHICH GLANDS WERE TAKEN		CONTROLS	
Total length	Hind legs	Total length	Hind legs	Total length	Hind legs
mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
80	8.5	81	8.5	81	8.5
82	10.0	82	8.5	82	9.0
83	9.0	81	9	80	8.0
80	8.5	80	10	79	8.5
81	8.5	83	8	81	10.0
82	9.0	79	8.5	83	8.5
81.3	8.9	80.7	8.8	81	8.8

TABLE 10

January 30, 1921

ENGRAFTED		CONTROL	
Total length	Hind legs	Total length	Hind legs
mm.	*mm.*	*mm.*	*mm.*
80	25	80	9.5
82	24	81	10.0
80	24	82	10.0
83	23	81	10.5
82	26	83	10.0
81.4	24.4	81.4	10.0

Note: One engrafted animal died January 23rd.

TABLE 11

March 1, 1921

ENGRAFTED		CONTROL	
Total length	Hind legs	Total length	Hind legs
mm.	*mm.*	*mm.*	*mm.*
83	31	82	13
82	32	83	13
82	29	81	14
84	30	82	12.5
82.7	30.4	82	13.4

One engrafted animal died February 25th. The experiment was abandoned March 10th, as some of the transplanted individuals had fore limbs. The data clearly indicate that the thyroid glands of neotenous tadpoles with hind legs 8 to 10 mm. are physiologically active and capable of inducing acceleration of limb growth and development when transplanted into other neotenous animals of similar size and developmental stage. However, metamorphic change following grafting is not so rapid or marked as when the same type of gland is implanted in immature (non-neotenous) larvae. But in either case the absorption of the secretion of the grafted gland induced a greater reaction than the same gland is capable of producing (in the same time and under similar conditions) when left unmolested in the neotenous animal of which it was originally a part.

SUMMARY OF CONCLUSIONS

1. Adult Necturus were fed, injected, and engrafted with physiologically active thyroid substance with negative results. The injection of 20 mg. of thyroxin iodine had no effect upon metamorphosis, whereas a 1 to 50,000 solution of this compound readily metamorphosed immature larvae of Amblystoma. The experiment indicates that perennibranchiate amphibians are permanent larvae and have lost the ability to transform under the stimulus of thyroid treatment.

2. Necturus differs markedly from the axolotl in its reaction to thyroid administration, as the latter readily metamorphoses when fed or injected with this substance.

3. The thyroid glands of the perennibranchiate Necturus are quite variable in size, and in some animals may occur as large vesicles more or less isolated from one another. In other individuals the glands are rather small and may consist of but four to six extremely large vesicles. In Necturus the thyroids are generally located near the apex of the triangle formed by the geniohyoid and external ceratohyoid muscles.

4. Despite its larval characters, Necturus possesses thyroid glands of great physiological activity, as shown by heteroplastic transplantation into immature anuran larvae.

5. A giant axolotl, 14.25 inches long, several years of age, was found to have a highly active metamorphosis-inducing thyroid apparatus. The thyroid of a single specimen cut into six pieces and transplanted metamorphosed five immature anuran tadpoles within two weeks. The sixth animal died following the operation.

6. The axolotl's thyroid is normal in appearance and of large size, consisting of numerous large vesicles filled with colloid. The gland is surrounded by a rich network of capillaries.

7. The failure of the axolotl to metamorphose appears to be due to the inhibition or the defective development of some unknown factor which normally serves to release the fully formed hormone from the thyroid into the blood stream. It is suggested that defective nervous stimulation or perhaps inhibition is the immediate cause of retention of the secretion within the thyroid vesicles, but that in the last analysis some defect of interrelation of the various components of the endocrine system is probably responsible for the nervous inhibition or lack of normal stimulation.

8. Experiments on large neotenous anuran tadpoles indicate that the failure of these animals to metamorphose at the proper time probably is due to the same causes responsible for axolotl neoteny: i.e., the thyroid glands apparently do not secrete their fully formed hormone into the blood stream because of some unknown inhibiting influence. The thyroid inhibition seems to be less marked in anurans than in the axolotl, since neotenous tadpoles eventually metamorphose if given sufficient time.

9. The next step in the analysis of amphibian neoteny is to determine the nature of the factor responsible for the failure of the thyroid to release its hormone (or at any rate to render it impotent in so far as metamorphosis is concerned). Is this unknown factor hormonal, or nervous, or both?

10. In the older work on amphibian neoteny too much stress was laid upon the exogenous factors as causative agents, and too little, if any at all, upon endogenous factors, and heredity.

ADDENDUM

Uhlenhuth's claim that urodeles differ from anurans in that their metamorphosis is independent of iodine and influenced only by the thyroid hormone itself is rendered invalid by the following experiment: The thyroid glands of large axolotls (seven inches total length) were extirpated and the animals kept for five months in the laboratory following the operation, then injected twice with strong doses of tyrosine in which two atoms of iodine had been substituted for two hydrogen atoms of the molecule forming the compound 3-5 diiodotyrosine. The animals metamorphosed within seventeen days following injection. Control thyroidless axolotls injected with equal quantities of pure tyrosine and later with large amounts of 3-5 dibromtyrosine showed no evidences of metamorphosis.

Further evidence that Uhlenhuth's claim is not valid is furnished by Huxley and Hogben who metamorphosed Salamandra and Triton larvae by immersion in dilute solutions of iodine (Proc. Roy. Soc., vol. 93, 1922); by Hirschler, who metamorphosed axolotls and tadpoles by administration of elemental iodine and iodoform (Arch. Entw. Mech., 1922), and lastly by Jensen who metamorphosed axolotls by injections of iodized casein, iodized serum globulin and iodized serum albumen (Compt. Rend. Soc. de Biol., 85, 391–392, 1921).

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PLATE 1

EXPLANATION OF FIGURES

3 Larva grafted ten days with small piece of Necturus thyroid, and control.

4 and 5 Larvae engrafted eight days with pieces of Necturus thyroid.

6 Larva grafted six days with Necturus thyroid, and normal control.

7 Larva fed mammalian thyroid tissue (desiccated) eight days. Control animal same as in figure 6.

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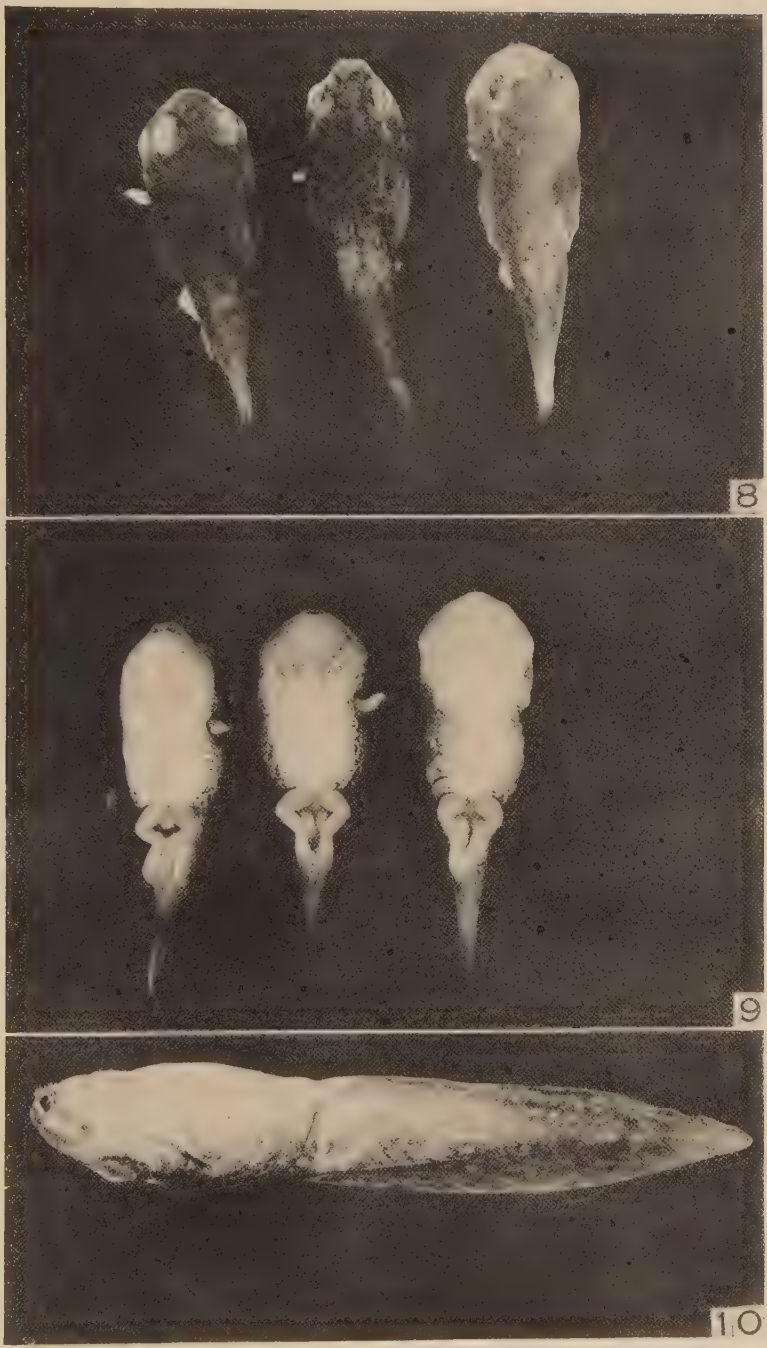
PLATE 2

EXPLANATION OF FIGURES

8 Immature R. clamata larvae ten days following transplantation small pieces of axolotl thyroids.

9 Same as figure 8. Ventral view.

10 Control larva for animals shown in figures 8 and 9.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SPONTANEOUS METAMORPHOSIS OF THE AMERICAN AXOLOTL

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The following experiments on axolotl neoteny and metamorphosis are published, not because of the conclusive nature of the results obtained, but the reverse—because of their inconclusiveness. A record of the work seems warranted in order that other investigators of this problem may be spared considerable expense, time and effort due to unsuitability of the material for experimentation.

The latter part of April, 1922, one hundred and nine axolotl larvæ of *Amblystoma tigrinum* were received from Albuquerque, New Mexico. These animals were obtained through the courtesy of Mr. J. N. Gladding. They varied in length from four inches to fourteen inches, though the average total length was about seven inches. One animal measured fourteen inches from snout to tail tip, another measured eleven inches. They were the largest individuals of the lot. The animals were in excellent condition on arrival and none showed any indications of metamorphosis.

Experiment 1. Autoplastic Thyroid Transplantation

May 5, 1922, the thyroids of seven axolotls, seven inches in length, were removed under chloretone anesthesia and each gland transplanted intraperitoneally into the same individual from which it was taken. The idea was that the acquisition of a new blood and nerve supply by the gland in its new environment might permit the release of the accumulated secretion and so metamorphose the animal. It was shown by the writer ('21) that the thyroid glands of axolotls are highly active metamorphosis-inducing agents providing the hormone escapes into the

blood stream. In these forms there appears to be some inhibition of the secretory (excretory) functions of the thyroid, and the hormone is retained within the gland vesicles.

The experimental animals and their controls were kept in large aquaria with plenty of water and food. One of the grafted animals had metamorphosed by June 27. Three others transformed by July 1; a fifth animal died without transforming July 6. The two remaining axolotls had not metamorphosed by September 1. During the interval between May 21 and September 1, all of the controls spontaneously transformed. The experiment is, of course, without significance because of the unstable nature of the control material. It is highly probable that the operated animals would have metamorphosed just about as rapidly if the thyroid had been left in its normal position.

Experiment 2. Homoplastic Thyroid Transplantation

Five seven-inch axolotls were engrafted intraperitoneally with the thyroid gland of other animals of similar size and appropriately controlled by animals transplanted with pieces of muscle tissue.

The transplants were made May 2, 1922. One animal had transformed by June 3, a second by June 6, a third June 11. Two animals remained as larvæ and were reengrafted June 11 with axolotl thyroids, and metamorphosed by July 3. In the meantime the controls also transformed. A large series of transplantation experiments were performed, using various endocrine glands, but in every case except two experiments the controls metamorphosed along with the operated individuals.

Heteroplastic Thyroid Transplantation

Four eight-inch axolotls were engrafted intraperitoneally with the glandular tissue of adult *Necturus maculatus*. Each axolotl received the entire thyroid of a single

Necturus. The experiment was performed May 15. By June 11 all of the engrafted animals had transformed but none of the controls for this particular group, though normal, untreated animals used as checks for other experiments were metamorphosing during this interval.

Despite the unstable nature of the control material used, this experiment seems fairly sound and indicates that *Necturus* thyroids when injected in sufficient quantity will metamorphose axolotl. To be absolutely reliable this experiment should have been performed upon thyroidectomized forms, but unfortunately the unsuitable nature of the controls was not known until too late.

Thyroid Feeding Experiments

Five six-inch axolotls were fed desiccated thyroid tissue (Parke, Davis and Company), containing 0.21 per cent. iodine by weight. The feeding was done by means of a pipette May 18. Two animals had transformed by May 27, and all by June 10. None of the controls metamorphosed during this interval but all transformed by July 25. The experiment seems trustworthy, especially in view of similar results obtained by other investigators on animals of the European strain.

Heteroplastic Pituitary Transplantation

Five axolotls varying in length from four to seven inches were each grafted with two whole pituitary glands of adult *Rana clamata* frogs. The grafts were made May 5. June 3 one animal metamorphosed; June 7 a second transformed. June 10 the three remaining animals were reengrafted with frog pituitaries. All metamorphosed by June 25.

During the interval between May 5 and June 25 only two of the controls for this particular group transformed, but it must be remembered that control animals of other cultures were metamorphosing. The experiment is recorded for what it is worth, but the writer believes that

injection of fresh pituitary substance does induce axolotl metamorphosis possibly by serving to release the thyroid hormone. This experiment should be tried on the Mexican strain of axolotl which apparently rarely spontaneously metamorphoses and hence can be safely controlled.

Thyroidectomy and Metamorphosis

Eight axolotls varying from seven to fourteen inches were thyroidectomized and at the present writing, September 1, are still larvæ and show no indications of transforming. Out of the original one hundred and nine animals received from New Mexico these eight are the only ones that have not metamorphosed. It is a fairly safe assumption that these axolotls will remain permanently as larva now that the thyroid gland is lacking.[1]

The thyroids of several animals were removed after the onset of metamorphosis, *i.e.*, after the tail fin and gills were undergoing reduction, but in all cases the removal of the thyroid failed to prevent the completion of metamorphosis.

Discussion

The conclusion to be drawn from these experiments is that the New Mexican strain of axolotl is entirely too unstable to work with on any problem involving the methods of feeding, injection or transplantation, where the results require a lapse of several weeks to obtain. The animals can not be controlled when the thyroid apparatus is left intact. It is evident that conclusive experiments of the above kinds on the New Mexican strain of axolotl (where the animals themselves are used as

[1] The thyroidectomized animals were kept for five months and then injected with iodotyrosine and iodoserumglobulin. Metamorphosis resulted within a period of twenty days following injections of either substance. Two partially thyroidectomized animals which had failed to transform were metamorphosed by injection of iodoserumglobulin. Injections of tyrosin, dibromtyrosin and globulin had no effect upon metamorphosis. Uhlenhuth's conclusion that only thyroid iodine (iodine which has undergone transformation within the thyroid gland) is effective in metamorphosing urodele larvæ is invalid.

experimental material) can only be obtained by using thyroidectomized animals.

Professor Henry Laurens, of the Department of Physiology, informs me that several years ago he had a similar experience with axolotls from New Mexico. He received a shipment of several dozen in the spring, but was unable to prevent them from transforming shortly after arrival in New Haven. Only one animal of the lot failed to metamorphose and was kept two years in the laboratory, attaining a length of 14.25 inches. This individual was used by the writer for thyroid transplantation work.

The marked tendency of the New Mexican and other American axolotls to metamorphose spontaneously when moved from one locality to another prevents their being used for aquarium purposes. It is an odd fact that practically the only axolotls used as aquarium material in the United States are those that have been shipped from Europe.

The European strain seems to differ from the New Mexican form in regard to spontaneous metamorphosis, because these animals are handled by practically all aquarium dealers in Germany and can be obtained for a few cents apiece. Apparently they rarely spontaneously transform according to Jensen ('20), who has worked extensively with this strain. The curious thing about the New Mexican strain is that in their native habitat they too may remain for considerable periods as larva, yet when shipped from New Mexico to New Haven promptly metamorphose regardless of size or age. One large animal of this strain obtained by Professor Laurens failed to transform and was kept in the laboratory for two years; at the end of this time it showed no indications of metamorphosis and was killed for thyroid transplantation work.

According to Gadow ('08) the strain of axolotls established in Europe came originally from the vicinity of Mexico City. The first axolotls were brought to France by Marshal Forey in 1863, and the present strain is de-

scended from these animals. Gadow also states that the axolotls of Lake Xochimilco have never been known to metamorphose in their native habitat. However, several of the descendants of the animals taken to Europe did metamorphose, so that spontaneous transformation in the Mexican strain does sometimes occur, though rarely.

In an earlier paper ('22) the writer showed that the thyroid mechanism of axolotls is filled with physiologically active hormone capable of inducing metamorphosis but that the secretion is apparently not liberated into the blood stream, hence the retention of the larval characters despite the possession of a large well-formed gland. The thyroid of a fourteen-inch axolotl several years of age was extirpated and cut into small pieces, each piece then transplanted into an immature Anuran larva. The single axolotl thyroid promptly metamorphosed five such tadpoles within fourteen days, whereas left intact within the axolotl's body it was quite incapable of inducing transformation.

This same experiment was repeated upon thyroidectomized and hypophysectomized *Rana sylvatica* tadpoles with similar results. Small pieces of axolotl thyroid when engrafted into thyroidless and pituitaryless larvæ promptly induce metamorphosis within ten or twelve days.

It is quite clear from these experiments that axolotl neoteny is due to retention of the thyroid hormone within the gland vesicles. Under normal conditions and in its native habitat, the releasing mechanism apparently fails to act, but when the animals are shipped from one place to another and subjected to new environmental conditions metamorphosis promptly ensues. In the New Mexican strain slight stimulation is sufficient to initiate metamorphosis, but in the European and Mexican forms very powerful stimulation is needed to overcome the thyroid inhibition and release the secretion. In the European strain the following agents have been used successfully for inducing metamorphosis: thyroid feeding (Laufber-

ger '13), salicylic acid injections (Kaufman '18), iodine and iodoform injections (Hrischler '18–'19), organic iodine feeding—iodothyrosine, also injections of iodocasein, iodoserumglobulin and iodoserumalbumin (Jensen '21); and of course Marie von Chauvin's experiments are well known.

It is evident that the peculiar thyroid inhibition causing neoteny in axolotl is due to genetic factors and that the condition is hereditarily transmitted. It is interesting to note that in axolotl we have one of the best examples of hereditary transmission of an endocrine defect known. Attempts to explain neoteny by assuming that environmental agencies such as cold, altitude and the like are the chief causative factors are too crude to be seriously considered and for this reason—the aquarium dealers of Europe breed their animals as larvæ and the young grow up as axolotls, the matter of cold or altitude not entering into the question. As was previously mentioned, the European strain arose from a few animals taken to France in 1863.

Then, too, both Professor Laurens and myself received our animals from Albuquerque, New Mexico, where they breed. The animals were old when captured. The temperature of the pools in the vicinity of the city can not be very low even in winter—not nearly so cold as those of the middle western states, northern New York, Ohio, or Wisconsin—and axolotls have never been reported as occurring in these states so far as the writer is aware.

The *Amblystoma tigrinum* resulting from the metamorphosis of the axolotls during my experiments were placed in certain pools in the vicinity of New Haven where other species of *Amblystoma* are known to breed. The animals are full grown and should breed next spring (1923). By following the life history of the larvæ it is hoped that some new light may be shed upon the obscure and much debated problem of the relation of neoteny to environment.

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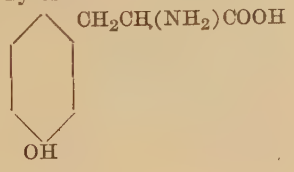
IODINE AND ANURAN METAMORPHOSIS

THE following experiment shows clearly the importance of iodine in inducing anuran metamorphosis and indicates that other halogens such as bromine can not be substituted for it.

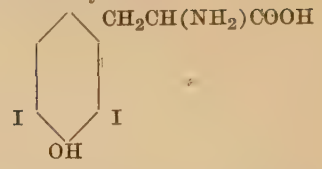
Thyroidectomized and hypophysectomized *R. sylvatica,* the glands of which had been extirpated early in embryonic life, were kept until after the normal time for metamorphosis had elapsed and the normal controls had transformed, and then divided into three sets for experimental purposes. It will be recalled that thyroidless and pituitaryless larvæ do not metamorphose but remain permanent tadpoles unless fed thyroid derivatives or very large amounts of iodine. One set of animals was fed quantities of pure tyrosine $CH_2CH(NH_2)COOH$ each day and allowed to remain in weak solutions of this substance six to eight hours every day. The remainder of the time they were kept in large glass containers and fed large quantities of spirogyra.

The second lot of animals was fed equal quantities of tyrosine in which two atoms of iodine had been substituted for two hydrogen atoms of the tyrosine—forming the well-known compound, 3-5-di-iodo-tyrosine. The iodine in this compound is, of course, an integral part of the molecule.

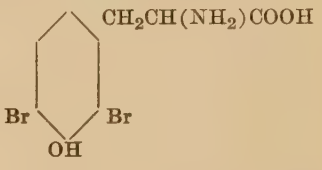
Tyrosine



3-5-diiodotyrosine



The third set of tadpoles was fed quantities of tyrosine equal in amount to that received by the other two cultures, in which two atoms of bromine had been substituted for two hydrogen atoms of the tyrosine molecule forming the compound 3-5-di-brom-tyrosine.



The di-brom-tyrosine was prepared according to the method of C. T. Mörner, 1913, *Zeitschrift für physiologische Chemie,* Vol. 88.

The animals fed tyrosine and spirogyra showed no changes indicative of metamorphosis after sixty days of continuous feeding, though the normal growth rate was not interrupted. After the twenty-fifth day the hind legs differentiated and grew very slowly until they attained a length of 3 to 5 millimeters. There were no signs of fore legs, skin autolysis in the pectoral region, tail shrinkage or anything suggesting transformation.

The animals fed 3-5-di-brom-tyrosine behaved in identical fashion with those of the tyrosine-fed culture and no metamorphosis resulted. At the end of the second month of feeding the hind legs of the animals of the culture averaged five millimeters. The growth rate of the animals was not interfered with by the brom-tyrosine compound. Insofar as the acceleration of metamorphosis is concerned, the results obtained by feeding

tyrosine and 3-5-di-brom-tyrosine are essentially negative.

Conversely the administration of 3-5-di-iodotyrosine to thyroidless and pituitaryless larvæ brought about very striking results, and in so far as metamorphosis is concerned, simulated the action of thyroid extract, although the effect of the latter is somewhat more rapid. Within a few days after feeding iodo-tyrosine the animals appear thin and emaciated; the limbs grow rapidly and the other changes incident to metamorphosis appear. Twenty days after the date of first feeding the entire culture of thyroidless animals had completed metamorphosis except for the loss of the tail. The pituitaryless animals developed fore and hind legs, frog mouths but invariably died before tail resorbtion was complete. In two pituitaryless animals the right fore legs broke through the skin eight days after the animals were placed upon the iodo-tyrosine diet. These two individuals were exceptional in this respect, the average time being about twenty days.

These results are of significance for several reasons: 1. The experiment is clear cut and admits of but one interpretation, *i. e.,* that it is the iodine within the tyrosine molecule that is essential for Anuran metamorphosis, because tyrosine without the two atoms of iodine is incapable of inducing metamorphosis.[1]

[1] Large axolotls seven inches long were *thyroidectomized* and kept five months following the operation and then twice injected with iodotyrosine. Metamorphosis resulted within seventeen days following the first injection. Similar animals injected with tyrosine and dibromtyrosine did not transform. Partially thyroidectomized axolotls (two thirds of the gland excised) were kept five months and then twice injected with iodo-serum globulin. Metamorphosis occurred within sixteen days. Uhlenhuth's claim that Urodele larvæ differ from Anurans in regard to metamorphosis and iodine is not sustained.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 12

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THE PROSPECTIVE SIGNIFICANCE OF THE CELLS CONTAINED IN THE FOUR QUADRANTS OF THE PRIMITIVE LIMB DISC OF AMBLYSTOMA[1]

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ELEVEN FIGURES

It has been shown by Harrison ('18) that the limb-forming tissue in the tail-bud stage of the embryo of Amblystoma punctatum is in the form of a flat plate of mesoderm cells located just under the third, fourth, fifth, and half of the sixth somites and just ventral to the pronephros. Since the limb at this stage of development is an equipotential system—any part being capable of forming any other part or even the whole limb—it is not possible by simple extirpations to determine the respective contributions of the different regions to the definitive limb in normal development. By experiments with half discs Harrison found that the potencies of the limb-forming tissue are not equal throughout the disc, but are greater in the anterior and dorsal halves. Figure 1 shows the limb area divided into approximately equal parts as regards limb-forming power. It was surmised from observations on normal development that "the distal part of the limb is developed more particularly from cells lying in the posterior half, the ulnar half arising from the dorsal and the radial from the ventral quadrant." Attempts to determine the fate of the various regions of the disc by implantation of markers gave negative results.

[1] This work was reported at the New Haven meeting of the American Association of Anatomists, 1921, and an abstract appears in the Proceedings (Anat. Rec., vol. 23, no. 1, p. 40). Further experiments have necessitated a few changes in the conclusions set forth at that time. These changes are embodied in the present communication.

The prospective significance of the cells contained in the primitive limb disc becomes of particular interest in connection with the relationship between the extent of the rudiment and the formation of the brachial plexus and the innervation of the limb (cf. especially Harrison, '07, and Detwiler, '22). The present investigation demonstrates the normal fate of the material in the different quadrants of the limb disc.

The writer wishes at this time to express his appreciation of the valuable advice received from Prof. Ross G. Harrison during the completion of this study.

EXPERIMENTAL

Another attempt was made to place markers in various places under the ectoderm of the limb area with the intention of locating them later by sectioning. Bits of notochord removed from another animal and stained in a dilute solution of Nile-blue sulphate were used for this purpose, the stain persisting long enough for the position of the implanted fragment to be observed during several days subsequent to the operation. By daily observations on these animals it was seen that the only shifting in position of the marker which took place was the slight change caused by wound healing; the limb grew out normally and left the transplanted bit of notochord in the body wall at the base of the limb. Obviously, this method is not adapted for determining the point in question.

The technique which gave the best results consisted in the use of the intra-vitam stain directly on the limb-forming cells themselves and observation of their distribution throughout development. By heavily overstaining the embryos in Nile-blue sulphate (overnight in a 0.003 per cent solution) the mesoderm cells may be made to take up the excess of stain which penetrates the ectoderm. The latter is, however, badly injured by the procedure and in the course of a day or so sloughs off—a circumstance naturally resulting in the death of the embryo. The mesoderm, on the other hand, is not injured, and if covered with unstained ectoderm and transplanted to an unstained embryo, will go on in development in a perfectly normal fashion.

Two variations in the method of operation were used, and while both gave identical results, the one described below is considered the better for a variety of reasons. Amblystoma larvae in stage 27 were heavily overstained in Nile-blue-sulphate solution. Then the ectoderm of an area somewhat larger than the limb disc (4 to 7 somites) was removed from an animal so stained and immediately replaced by normal ectoderm to obviate the difficulties imposed by the deleterious action of the stain on the ectoderm. In this operation care was taken not to injure either the blue mesoderm or the ectoderm used to cover it. In the course of a few hours healing was complete, and then the second step in the operation was carried out. This consisted in the

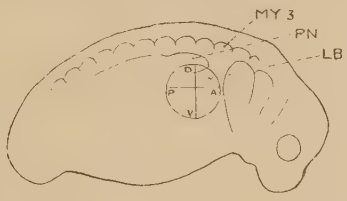


Fig. 1 Embryo of Amblystoma punctatum in the tail-bud stage (stage 29), showing the location of the limb-forming cells (*LB*). The vertical and horizontal lines crossing the area divide the disc into approximately equal parts as regards limb-forming power. *MY 3*, the third myotome; *PN*, pronephros; *LB*, limb bud. (From Harrison, '18, p. 418, fig. 2.)

transplantation of a portion of the limb disc which had been covered with normal limb ectoderm, from the Nile-blue animal to a corresponding position recently prepared in an unstained embryo. This latter animal, thus having part of the limb mesoderm stained blue, was kept for observation. The color remained visible during a period of seven or eight days, or until the bifurcation demarcating the first digits appeared at the tip of the limb (stage 41). It is at about this time that differentiation of the muscular and skeletal elements begins in the limb blastema, and the stain in no case persisted after this stage of development. This was, however, long enough to show definitely the distribution of the blue mesoderm to various parts of the limb, inasmuch as the flexor and extensor surfaces and the pre- and post-axial borders may readily be distinguished at this time.

The experiments are grouped in series according to which part of the limb disc was replaced by stained tissue. They may best be considered under the separate headings.

ANTERIOR HALF[2] STAINED (SERIES ALM)

The series includes experiments in which the anterior half of the limb disc with the stained mesoderm was transplanted in normal orientation to an unstained animal (fig. 2, A). The total number of positive experiments in this series is twelve and the limb formed on the operated side was normal in each case. With the outgrowth of the limb there was a rearrangement of cells in the stained half of the disc and a pulling out of the blue cells on the anterolateral side of the bud. The colored area at this stage (stage 38–39) was generally in the form of a triangle the base of which was slightly distal to the most anterior prominence of the limb and the apex just proximal to the tip (fig. 2, B). Not all the blue cells went into the limb, however, some remaining anteroventral to its base and contributing to the structures of this region (cf. Detwiler, '18). As the limb further elongated and became flattened dorsoventrally, the apex of the blue triangle was drawn out and at the time of the last observation before the disappearance of the color, its distribution to the free extremity was mainly confined to the lateral side of the base of the limb, encroaching also somewhat on the dorsum (i.e., the extensor surface), with a fine stream of cells running out on the radial side to the tip of the limb (fig. 2, C).

DORSAL HALF STAINED (SERIES DLM)

In this series the dorsal halves of limb discs with blue mesoderm were transplanted in nine cases (fig. 3, A). Of these, one died at an early stage from edema, three developed limbs which showed slight abnormalities, and two reduplicated as a consequence of the operation. The three remaining animals formed normal limbs. In these three latter cases, as also in the abnormal ones,

[2] In the later experiments the cuts dividing the limb disc into 'halves' were made somewhat excentrically, thus corresponding more exactly with the divisions shown in figure 1.

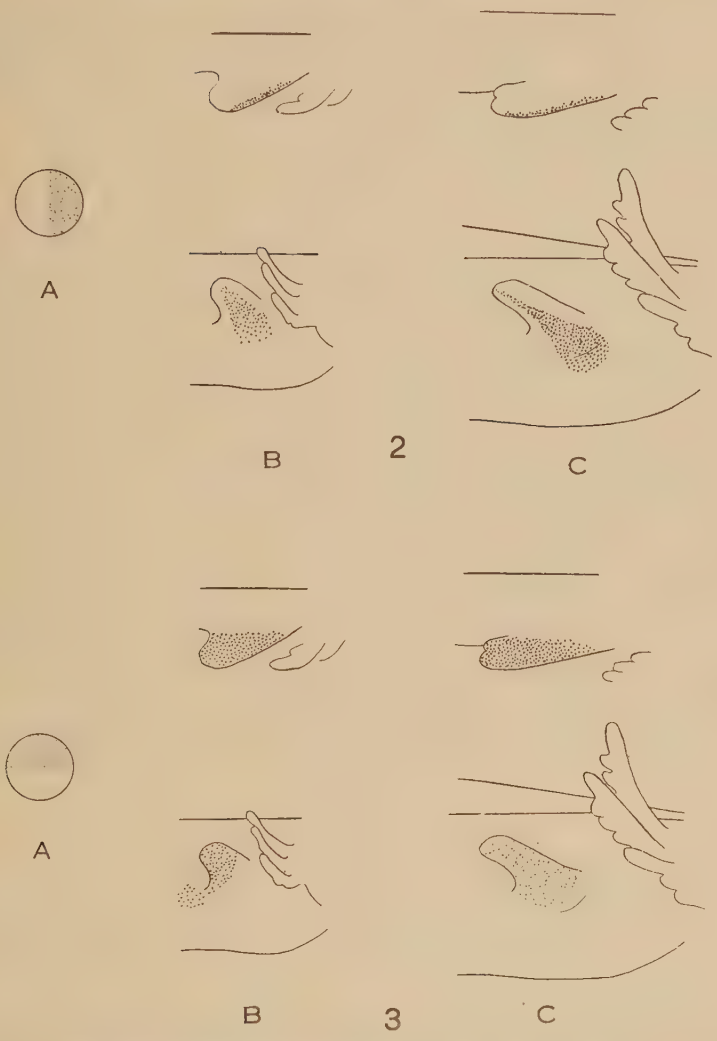


Fig. 2 Semidiagrammatic representation of the distribution of the cells from the anterior half of the limb area (represented as of the right side of the body). A. The limb disc in stage 29 with the mesoderm of the anterior half stained blue, denoted by stippling. B. Dorsal (above) and lateral views of the limb bud, stage 38–39, showing regions where the stained cells are present. C. Dorsal (above) and lateral views of the limb, stage 40–41, showing distribution of the stained cells at the time of the disappearance of the color.

Fig. 3 A, B, and C, same as figure 2, except here the dorsal half of the limb area is composed of stained cells.

the stained area covered the entire limb bud when it first appeared and remained so distributed as long as the color was visible. Its occurrence on the extreme anterolateral prominence of the shoulder is doubtful (fig. 3, B, C).

POSTERIOR HALF STAINED (SERIES PLM)

This series includes seventeen embryos in which the posterior half of the limb disc, stained with Nile blue, was transplanted (fig. 4, A). In twelve of these cases a perfectly normal limb resulted; the other five were very nearly normal, the only abnormality being a very slight shortening of the appendage as a whole. In each of these cases as soon as the limb bud became visible, the blue cells covered its posterior half, and when the tip had begun to point definitely, the blue area was seen as a distinct cap covering the distal part of the limb on all sides and extending along the posteroventral surface on to the body wall to a greater or less degree (fig. 4, B). At stage 40 the blue was seen to cover the flexor surface of the limb from the shoulder to the tip, and to bend dorsally over the latter, still giving the impression of a cap at the end of the limb. The distribution to the dorsal (extensor) surface was, however, slight, being limited to the region of the future hand and forearm (fig. 4, C).

VENTRAL HALF STAINED (SERIES VLM)

The seven animals in this series had the mesoderm of the ventral half of the limb disc stained in Nile blue (fig. 5, A). In each case the resulting limb was normal. When the bud first appeared the blue area was entirely ventral to it, but at about stage 38 the most anterior prominence at the base of the bud was seen to be formed from the blue cells (fig. 5, B). Most of the cells, however, remained in the body wall and took no part whatever in the formation of the limb. Later stages (stage 40–41) showed the blue covering the most anteroventral prominence of the shoulder, extending out more or less on the radial border of the limb and with a wide distribution to the body wall ventral and slightly anterior to the base of the limb (fig. 5, C).

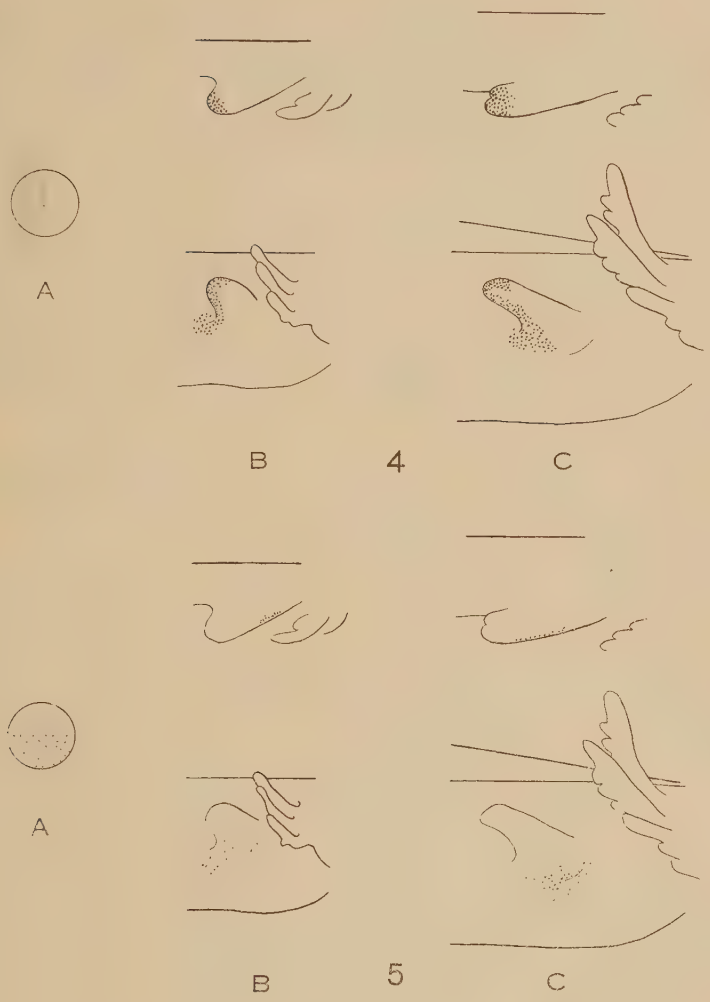


Fig. 4 A, B, and C, same as figure 2, except in this case the posterior half of the disc is stained.

Fig. 5 A, B, and C, same as figure 2, except here the ventral half of the disc is stained.

COMPARISONS

By a combination of the portions of the definitive limb which were stained when dorsal and ventral halves were transplanted (figs. 3, C, and 5, C) the whole limb is seen to be covered, but a combination of the anterior and posterior halves (figs. 2, C, and 4, C) leaves a considerable area of the extensor surface unaccounted for. Since it is known that some of the limb-forming cells are located underneath the pronephros, the possibility suggested itself that the uncovered area might be formed from these cells. Failure to include all of them in transplanting anterior and posterior halves would not be impossible because of the technical complications imposed by the proximity of the pronephros. Two further series of experiments were devised to test this possibility.

PORTION OF RUDIMENT COVERED BY PRONEPHROS STAINED (SERIES SLM)

In this series, which contains six positive cases, the technique was the same as before, except when transplanting to the unstained animal a horizontal cut was made through the limb just ventral to the pronephros and an area transferred which included both the pronephros and the tissues underneath and around it (fig. 6, A; cf. also fig. 1). Interference with the normal function of the pronephros caused considerable edema and in only two cases did the limbs approach normality. Both of these were slightly retarded in development, while the other four were quite small. All of them, however, showed a distribution of the stained cells only to the dorsum of the limb (fig. 6, C). In two cases the stain was limited to the ulnar side, in four it covered the entire extensor surface. One of the nearly normal limbs falls in the first group, the other in the second.

PORTION OF THE RUDIMENT NOT COVERED BY THE PRONEPHROS STAINED (SERIES OLM)

In this series the portion of the rudiment lying ventral to, and not covered by the pronephros was transplanted (fig. 7, A). The three positive cases showed a distribution of the stained

area to the entire flexor surface and a part of the radial border of the limb (fig. 7, C).

From these last two series it is obvious that nearly all of the extensor surface of the definitive limb is formed from cells which in the tail-bud stage are situated beneath the pronephros.

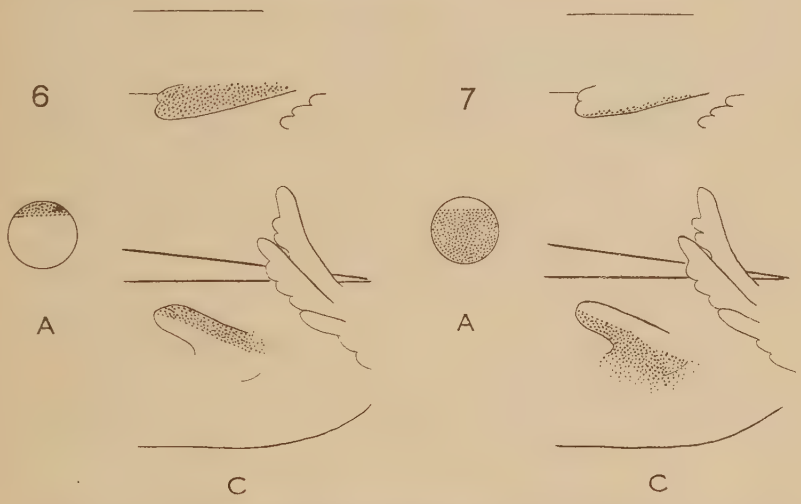
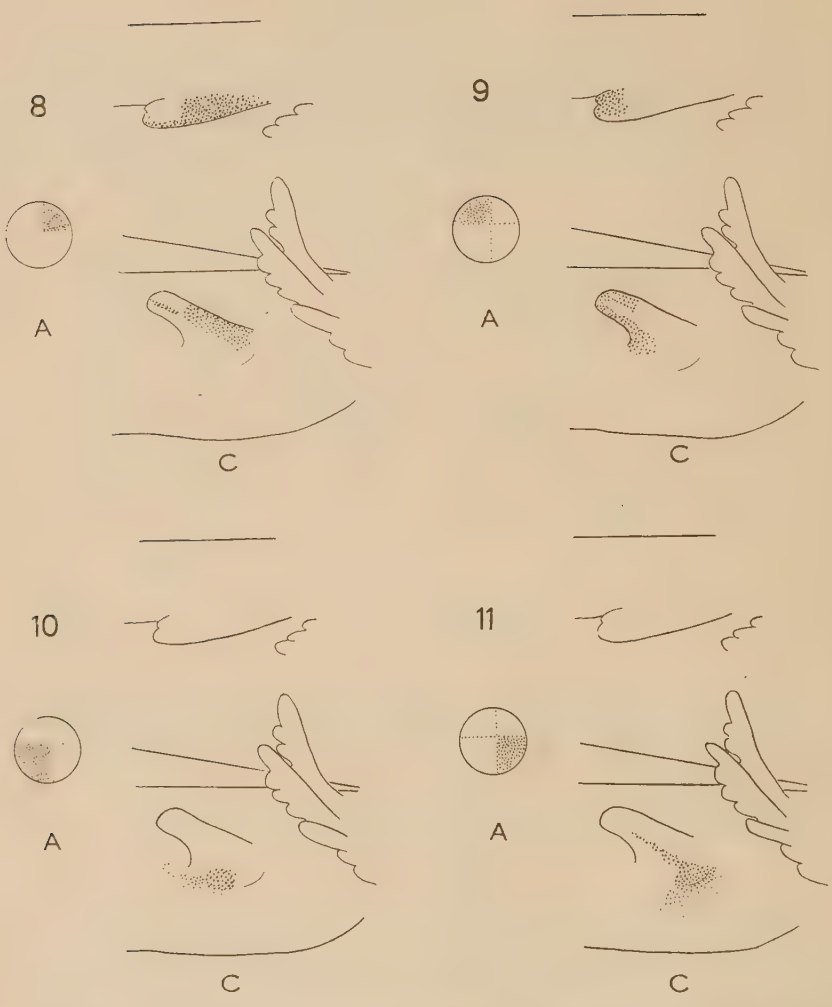


Fig. 6 A and C, same as figure 2, except here only those limb-forming cells are stained which are situated underneath the pronephros in the dorsal half of the limb disc.

Fig. 7 A and C, same as figure 2, except that all the cells of the limb disc which are not covered by the pronephros are stained.

CONCLUSION

These experiments furnish evidence substantiating the deductions made by Harrison from his observations on the normal development of the limb as well as from his experiments in transplanting half discs. It is seen that by no means all of the cells which are potentially limb forming do actually go into the limb. It is obvious that the mesoderm of the dorsal half of the limb disc forms the greater part of the limb with the anterior half second in importance. Of the cells in the dorsal half of the disc, those situated under the pronephros in the tail-bud stage form practically all of the extensor surface of the definitive limb,



Figs. 8 to 11 Diagrammatic representations (C) of the portions of the limb (stage 40–41) formed by cells from the various quadrants of the limb disc of stage 29, shown in A by the stippled area (represented as of the right side of the body).

Fig. 8 The anterodorsal quadrant.
Fig. 9 The posterodorsal quadrant.
Fig. 10 The posteroventral quadrant.
Fig. 11 The anteroventral quadrant.

while those not covered by the pronephros are destined to produce nearly the whole of the flexor surface.

By superposing overlapping stained portions (e.g., figs. 2, 3 and 6; figs. 3, 4, and 6, etc.) the contributions of the various quadrants have been determined. Figures 8, 9, 10, and 11 show diagrammatically the results of such a procedure. Summarized briefly, they are as follows:

1. The principal rôle of the anterodorsal quadrant is in the formation of a large part of the dorsal surface (proximal) and radial border of the limb, together with proximal parts of the shoulder (fig. 8).

2. The posterodorsal quadrant forms the distal part of the dorsum and all of the flexor surface of the limb (fig. 9).

3. The posteroventral quadrant contributes only slightly to the proximal part of the shoulder, most of its cells do not go into the limb at all (fig. 10).

4. The anteroventral quadrant helps to form the shoulder, particularly at the most anterior part, and sends a few cells out on the radial border of the limb. For the most part its cells remain in the body wall of the larva (fig. 11).

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THYROID TRANSPLANTATION AND ANURAN METAMORPHOSIS

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SEVEN FIGURES

The problem was to determine by experimental means, such as thyroid transplantation, the stage in larval development of anurans, when the thyroid becomes physiologically active and capable of inducing the somatic changes incident to metamorphosis. It was considered probable that such a study might shed additional light upon the reason for the extraordinary larval lives of certain frog species, and incidentally upon some of the factors involved in neoteny.

The writer has held the view for some time ('20) that the long duration of larval life of such anurans as Rana catesbeiana and Rana clamata is probably due, in part at least, to the extremely slow rate of thyroid-gland development in these two species. If the idea is correct that the length of larval existence of anuran species depends in part upon the rate of thyroid development and differentiation in the species, then by extirpating these glands and transplanting them into other animals at various larval stages and noting the effect upon metamorphosis of the grafted forms, it should be possible to determine the degree of physiological activity of the thyroid mechanism at various larval stages, providing food and environmental conditions were equal in control and experimental cultures.

The larval development of R. catesbeiana is the longest of any anuran known. This form normally requires two years of larval existence before transformation, and more rarely three years. The size attained by the mature larvae is relatively very large compared with other anurans. At Princeton, New Jersey, where the writer had an excellent opportunity to observe

this species in its normal habitat, the eggs are laid the latter part of May. One year the first eggs were observed May 25th; the following year no eggs were found until May 29th. Wright ('14) states that in the region of Ithaca, New York, spawning does not occur until the last of June or even in July. First-season tadpoles average in length 30 to 35 mm. and have no indications of hind limbs save microscopic epithelial buds; second-season specimens attain a length of from 75 to 95 mm., and rarely show developing hind legs; the mature larvae vary from 112 to 160 mm. A specimen in the zoological collection of this laboratory measures 170 mm. and is the largest tadpole the writer has yet seen.

While studying the germ cells of R. catesbeiana larvae, the writer observed that some of the mature tadpoles at or very near the time of transformation develop ripe spermatozoa in the testes. Ten very large tadpoles were caught at Princeton, New Jersey, that showed numerous sperm. All of the larvae were over 120 mm. in length, and had hind legs from 27 mm. to 47 mm. long. This condition in the mature bullfrog tadpole at metamorphosis is not a rare thing, but is frequently encountered in mature (two-year-old) larvae. Many tadpoles, however, do not show the presence of sperm before or at metamorphosis, owing to great prolongation of the first abortive larval sexual cycle culminating in destruction of most of the primordial germ cells and the consequent delay in the formation of the definitive germ cells and germ gland (Swingle, '21). Like the axolotl, the bullfrog's larval life may extend to the period of sexual maturity, i.e., maturity in so far as the possession of a few ripe spermatozoa in the male is concerned. Female larvae at metamorphosis and for a considerable time afterward are never mature sexually, so far as the writer's observations go, and 470 animals were examined. It is, of course, not necessary to section females to check this point.

The larval life of Rana clamata, while not nearly so prolonged as that of the bullfrog, is yet, nevertheless, very long indeed, when compared with other anurans. Wright who has made a very painstaking study of the life-history of various American

anurans states that the time required for the metamorphosis of the green frog appears to be from 370 to 400 days. He says: "In a small pond where the species first laid June 6, 1906 (this bunch being the only eggs laid in the pond during the season), the first records of transformation in 1907 were July 13, thus giving 373 days before transformation. In 1908 tadpoles were transformed July 21, or 400 days after the eggs were deposited. In 1910 eggs were laid in a certain pond June 9; and on June 29 of the succeeding year (1911) the tadpoles began to transform, 385 days later."

TABLE 1

SPECIES	LENGTH OF LARVAL LIFE FROM TIME OF EGG DEPOSITION	TOTAL LENGTH OF TADPOLE AT METAMORPHOSIS	BODY LENGTH AFTER METAMORPHOSIS
	days	*mm.*	*mm.*
R. pipiens	75–111	60–80	24.0
R. palustris	90–100	60–74	24.0
R. sylvatica	89–94	37–47	16.0
R. clamata	370–400	65–91	24.0
R. catesbeiana	720–800	120–165	53.0
Bufo lentiginosus	50–65	22–28	9.6
Hyla pickeringii	90–100	26–33	11.0
Hyla versicolor	45–60	40–50	16.0

Rana clamata has normally, then, a larval life of something over a year, and many individuals spend much longer than this as larvae. It is quite common to find numbers of full-grown tadpoles of this species, in late fall, buried in the mud and detritus of the pond bottoms, preparing to spend an extra winter as larvae, i.e., they are neotenous forms. No observations have been made regarding the sexual conditions of R. clamata larvae, but it is very doubtful if the larval period extends to the period of sexual maturity as it does in the case of some bullfrog tadpoles.

Table 1, made up in part from personal observation, but principally from Wright's ('14) figures of the time normally required for metamorphosis of various anurans in this vicinity, shows clearly the striking disparity of R. catesbeiana and R. clamata in this respect when compared with other forms. No

other species even approaches them in length of larval existence. Incidentally, the measurements of the animals are also interesting as indicating the great variation in size at transformation of individuals within the species. With the exception, then, of these two species, the remainder of the group undergoes transformation the same season they hatch; in the case of Bufo and Hyla versicolor, within two months or less from the date of spawning.

There seem to be no particular growth requirements or other developmental necessities for such unusual prolongation of the tadpole stage as exhibited by R. catesbeiana and R. clamata. Many larvae of both species (especially the bullfrog) attain full size before even developing limb rudiments, though this is somewhat unusual. On the other hand, the writer has observed small, immature larvae of both the green frog and bullfrog with remarkable limb development for such individuals. That these two species are really abnormal in regard to their length of larval existence is indicated by the very great frequency with which their larvae remain extra seasons as tadpoles instead of transforming at the usual time. Then, too, the fact that R. catesbeiana tadpoles sometimes develop sperm while larvae proclaims them as neotenous.

In the writer's opinion, the long-drawn-out larval life of R. catesbeiana and R. clamata, in comparison with other anurans, is anomalous, and represents a secondary and regressive (neotenous) development in these two species, for it is probable that their larvae originally metamorphosed the same season they hatched as do the tadpoles of other members of the anura.

The prolonged larval life of R. catesbeiana and R. clamata, but more especially the former, appears to be due: 1) To extremely slow development and differentiation of the thyroid apparatus; 2) in neotenous individuals to retention of the physiologically active hormone after its formation within the gland vesicles. In other words, in individuals with abnormally prolonged larval lives the thyroid activity is more like that of urodeles, the secretion is apparently not released as soon as formed, but accumulates and is released by some unknown factor not all at once, but within a comparatively short interval.

MATERIAL AND METHODS EMPLOYED

Despite the fact that previous thyroid grafting work had been performed with bullfrog material and considerable data gathered, it was decided to try similar experiments on Rana clamata, because of the abundance of tadpoles of this species in the vicinity of the laboratory. In some respects the larvae of the green frog were more favorable even than R. catesbeiana for thyroid-transplantation work—the limbs differentiate more rapidly and the growth rate is faster. For experiments concerned with the problem of metamorphosis, it has been the writer's experience that the tadpoles of these two anurans, because of their slow development, are far preferable to species with shorter larval lives, such as R. pipiens or R. sylvatica.

The exact age of the tadpoles is unknown, they all came from the same pool and were of diverse sizes. When brought to the laboratory, the smallest larvae measured 23 mm. from snout to tip of tail; the largest animal measured 93 mm. and had hind legs 27 mm. long. This animal is the largest member of the species the writer has yet seen. No effort has been made to calculate the exact age by using size or developmental stage as criteria, though doubtless this could be done with a fair degree of accuracy. The age of the tadpoles is of no great significance in these experiments; it is stage of development attained, as manifested by degree of growth and differentiation of the hind limbs, that is important.

In measuring the animals attention was focused chiefly upon the hind limbs and total length of the animal from snout to tip of the tail. Measurements of the body length, i.e., from nose to anus, and tail length from anus to tip of tail separately, are useless details because of the great individual variation among the animals in these respects. Limb length refers to length of the hind legs from the toes to junction of thigh with body, measured from the ventral surface. The fore limbs of anurans, as is well-known, develop along with the hind legs, but are covered by the operculum and are not visible until a late stage of metamorphosis. When they finally break through the skin, the animal is but a few days from the end of its tadpole career.

The procedure used in implanting the thyroids in either homoplastic or heteroplastic transplants is simple, the operation requiring but a few moments either for subcutaneous or intraperitoneal transplantation. The larvae receiving the graft were not fed for two days before using, and were then narcotized in a 1–10,000 chloretone solution, such as utilized by Harrison ('04) and his students for this kind of work. With a pair of fine-pointed scissors or spear-head needle a minute incision was made through the body wall or into the lymph sinus and the tissue transplanted. No asepsis is necessary beyond using clean water, containers, and instruments. The animals were not fed until twelve hours after the operation. In these experiments both homoplastic and heteroplastic transplants were used, though homoplastic grafts were chiefly employed, as they undergo resorption by the host less readily than the other type. In practically all cases the implanted tissue was resorbed after six or seven weeks, generally in a much shorter time. In the process of resorption, however, the secretion of the gland is liberated, and if the gland possesses a physiologically active hormone, its presence and degree of activity is manifested by appropriate metamorphic changes in the tadpole. The ultimate fate of the grafted tissue is of minor importance in the present experiment. We are principally concerned with the degree of physiological activity of the glandular secretion of the larval thyroids at the time of transplantation, and not with the viability of the implanted tissue. It had been previously established by the writer ('22) that, in so far as anuran metamorphosis is concerned, the thyroid hormone is just as effective when released from the disintegrating vesicles of a heteroplastically transplanted gland, as from the living tissue. This fact accounts for the success of heteroplastic grafts in frog larvae (see experiment 7).

HOMOPLASTIC THYROID TRANSPLANTS

Experiment 1

The thyroid glands of newly metamorphosed Rana clamata frogs (both glands) were transplanted intraperitoneally into nearly

full-sized tadpoles, averaging 53.3 mm. total length, with undifferentiated hind-leg buds 2 mm. long. The control tadpoles were the same size and developmental stage. The grafts were made July 14th. Nine days later, the engrafted larvae showed signs of hyperthyroidism, such as marked growth and differentiation of the hind limbs, body emaciation, shrinkage of subcutaneous fat deposits, and faint autolysis of the skin on the right side in the pectoral region where later the fore leg appears. The controls showed no change. Fifteen days following transplantation, the tadpoles were in advanced stages of metamorphosis; three animals had developed fore and hind legs, frog mouth, and two others had the right fore leg through the skin but still retained the larval mouth. Two of the most advanced larvae showed some tail resorption. Skin autolysis over the region of the fore limbs had been apparent for several days previous to the appearance of the fore legs.

July 31st, or eighteen days after receiving the thyroid grafts, all of the animals had fore legs and frog mouths; several showed marked tail atrophy and resorption. They could not be reared to the stage of complete tail resorption, but invariably died when in the final stage of transformation (see discussion of experiment 2).

Briefly summarized, the experiment shows that the thyroid glands of newly metamorphosed frogs when transplanted into immature tadpoles will bring about practically complete metamorphosis within the brief period of twenty days—in some instances fifteen or sixteen days. This experiment is of importance for the remainder of the present work because it is used as a standard with which to compare the rates of metamorphosis of other cultures. The time interval which transplanted thyroid glands of newly metamorphosed frogs require for inducing metamorphosis in immature tadpoles is the criterion by which the physiological activity of larval thyroids taken from tadpoles in different developmental stages is determined. For instance, if the thyroids of young larvae, engrafted into other larvae of similar size and developmental stage, fail to induce any indication of metamorphosis such as limb growth and development (one of the first manifestations of thyroid activity in anurans) in the

same time interval required by implanted thyroids of newly metamorphosed frogs to bring about practically complete transformation of similar larvae, then such larval thyroids are considered to be physiologically inactive in so far as metamorphosis is concerned.

In a series of earlier experiments performed during the winter months the writer used as a standard of comparison for determining the degree of physiological activity of larval thyroids the time interval, necessary for a single thyroid gland (from one side only) of an adult frog to induce metamorphosis in immature larvae. There are several objections to employing adult glandular material, so this standard was not used. It is interesting to know, however, that a single gland of an adult frog (from one side only) when transplanted into larvae 60 mm. total length and full grown in regard to size, but with undifferentiated hind legs 2 mm. (a neotenous form), will bring about practically complete metamorphosis (tail not completely resorbed) within twenty-eight days in midwinter at laboratory temperature (fig. 1). The larvae used were caught in midwinter, January 12th, from under three inches of ice, and were evidently typically neotenic forms; that is to say, were spending an extra winter as tadpoles beyond the usual period required for metamorphosis.

Experiment 2

The thyroid glands of metamorphosing larvae with both fore and hind legs, but showing no tail atrophy or resorption, were transplanted into larvae averaging 50.8 mm. total length, with undifferentiated hind-leg buds 2 mm. The average length of the metamorphosing animals from which the glands were taken was 64.5 mm., nose-anus length 24.1 mm., hind-leg length 32.6 mm. The control tadpoles were of the same size and developmental stage as the engrafted animals. The experiment was begun July 15th. On August 1st five animals had both fore legs through, but none showed tail resorption. The larvae were in the last stages of metamorphosis, but appeared to be unable to complete the process of tail resorption like the animals of culture I. In order to overcome the metamorphic stasis (a stage

in which thyroid-grafted or thyroid-fed larvae generally die when metamorphosis is greatly accelerated), the young frogs (in all except for the presence of the tail) were engrafted with the

Fig. 1 Rana clamata larvae. *A*, control; *B*, animal originally same size and stage of development as *A* twenty-eight days after transplantation with one-half thyroid gland of adult frog. × 2.

pituitary glands of completely metamorphosed frogs. The work of Smith ('20) had indicated that the pituitary gland was responsible, somehow, for the complete transformation of tadpoles, and working on this hypothesis, the attempt was made to

rear the engrafted animals in which metamorphosis was greatly hastened, to the stage of complete transformation by implanting this gland. All three lobes of the hypophysis were transplanted together, and the results obtained tend to confirm Smith's observation, though too few animals were used to stress this point. The engrafted animals, five in number, were able to overcome the metamorphic stasis and completely transformed seven days following grafting. Almost invariably thyroid-grafted animals die before complete transformation. The implantation of the pituitary apparently aided the animals to overcome the metamorphic stasis (figs. 2 and 3). Experimental data derived from five animals is too meager to draw any conclusions from; the facts are significant, however. The writer hopes to repeat this experiment on a large scale during the summer.[1]

Comparison of the time intervals required for inducing transformation up to the stage of metamorphic stasis (fore and hind limbs and partial tail resorption) between the thyroids of newly metamorphosed animals and those taken from animals in the midst of the process (experiments 1 and 2) show that the physiological activity of the thyroids of completely metamorphosed

[1] Later work on a larger number of animals shows: (1) that the response of thyroid grafted larvae to homoplastic pituitary grafts is extremely variable, (2) that the implantation of pituitary glands into thyroid-fed tadpoles where the metamorphic processes were very greatly accelerated completely failed to help the animal through the critical period, (3) that homoplastic grafting of two pituitaries of newly metamorphosed frogs into normal larvae gives variable results, a few individuals responding by limb growth and other metamorphic change, e.g., fig. 5, a majority of the grafted animals of the same culture remaining unchanged.

Fig. 2 Thyroid grafted Rana clamata larva at the stage of metamorphic stasis.

Fig. 3 Thyroid grafted larva, engrafted with the hypophysis of a newly metamorphosed frog, at the stage shown in figure 2. The response is variable.

Fig. 4 Immature larva eighteen days following implantation of the thyroids of a mature (though not neotenous) tadpole 68 mm. long, with hind legs 11.5 mm., fore legs not visible.

Fig. 5 Pituitary grafted larva and control after fourteen days. Thirty days after grafting the animal metamorphosed, the control remaining unchanged. This type of response is the exception and not the rule. See footnote 1.

Fig. 6 Larva grafted with the thyroid of an adult Diemyctylus. Photographed after fourteen days.

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3
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frogs is slightly greater than that of metamorphosing animals. The difference between them, however, is slight.

Allen ('19) reported that in Bufo there is an actual diminution in the size of the thyroids and of the colloid masses at the very time when the process of metamorphosis is most active. This was presumed to be due to the absorption of an unusually large amount of stored colloid at the time when it would prove most effective. According to Allen's findings, the thyroid glands of my newly metamorphosed frogs (not fed since the transformation) should be still smaller and contain less colloid than those transforming. On the contrary, however, in Rana clamata such glands are physiologically slightly more active than those of transforming animals, though the writer has noted slight diminution in the size of the thyroids following metamorphosis such as Allen described. The size of a thyroid, however, is no sure indication of the stage of its physiological activity; indeed hypofunctioning glands are generally enlarged, e.g., colloid goiter.

Experiment 3

Small, immature tadpoles averaging 45.8 mm. total length, with undifferentiated hind-limb buds 0.5 mm. were engrafted with the thyroid glands of animals identical in regard to size and developmental stage, and controlled by others of the same lot. These small tadpoles were caught in a pool in the vicinity of New Haven and brought to the laboratory July 12th, hence must have been one year of age and for some unknown reason retarded in growth because year-old larvae of Rana clamata are generally larger than 45.8 mm. at this period of the year and have considerably greater limb development. These animals could not have metamorphosed before the onset of winter and were destined to become typical neotenous tadpoles. The thyroid transplants were made July 14th. August 4th, control and engrafted animals were in the same stage of development; no effect upon growth or differentiation of the larvae was noted.

Summarized, the experiment shows clearly that transplantation of the thyroid glands of immature larvae with totally undifferentiated hind-limb buds into other similar-sized tadpoles

has no appreciable effect upon metamorphosis or rate of limb development within the same time interval required by the thyroids of newly metamorphosed frogs when transplanted to induce practically complete metamorphosis in similar animals. The experiment warrants the conclusion that the thyroid glands of such tadpoles are relatively physiologically inactive at this stage and contain insufficient internal secretion to accelerate metamorphic change. It would be odd if the thyroid glands of larvae without indications of limb development should, when transplanted, induce metamorphic change or limb growth in other equally immature individuals. There can be little doubt that in anurans the progress of limb development is directly correlated with and dependent upon a progressive differentiation of the thyroid apparatus. When the physiologically active hormone has reached a sufficient concentration in the organism, complete transformation occurs. Metamorphosis does not consist of a sudden throwing off of the larval characters and development of the terrestrial type of structure at some stage of tadpole life, but the various somatic changes are initiated gradually and progress slowly at first, becoming more and more rapid as the concentration of the hormone increases within the blood and tissues. The development of the limbs is a good illustration of the whole metamorphic process in anurans.

The first appearance of the undifferentiated epithelial limb anlagen is independent of thyroid activity, because such buds develop before the gland is sufficiently differentiated to be physiologically active. However, the further growth and differentiation of these microscopic epithelial buds depends upon the thyroid and its iodine-containing hormone. It is interesting to note also that thyroidless larvae whose glands have been extirpated at their very inception, nevertheless develop limb anlagen, though these structures cease to grow and differentiate in the absence of the gland. In salamanders limb growth and development is practically, if not entirely, independent of thyroid function. The writer called attention to this peculiarity some years ago (Swingle, '19). Axolotls may have perfectly developed fore and hind legs, yet the thyroid mechanism of these

animals is quite unable to transform the individual, owing to some unknown factor which probably prevents the release of the fully formed and physiologically active hormone into the blood stream.

Experiment 4

The thyroids of larvae 51.5 mm. long, with undifferentiated hind-limb buds 1 mm. were transplanted into other tadpoles of the same size and developmental stage. These animals were brought to the laboratory July 12th, and it is doubtful if they could possibly have undergone transformation before winter. July 14th, the tadpoles were grafted, and on August 4th practically no change had taken place in regard to metamorphic change or limb growth. Larvae with epithelial hind-leg anlagen 1 mm. long possess a thyroid mechanism practically undeveloped physiologically for within the same time interval that thyroids of newly metamorphosed frogs or metamorphosing animals induce practically complete metamorphosis, when grafted, into similar-sized tadpoles, the larval glands contain barely enough active secretion to stimulate limb growth 1 mm.—a negligible result. Table 2 gives the details of the experiment. The figures represent averages.

TABLE 2

DATE EXAMINED	ENGRAFTED ANIMALS		CONTROL ANIMALS	
	Average length	Hind legs	Average length	Hind legs
	mm.	*mm.*	*mm.*	*mm.*
July 14	51.5	1.0	51.9	1.0
July 22	52.5	2.3	52.6	2.1
July 28	53.0	2.8	52.5	2.3
August 4	54.5	2.9	53.5	2.3

Experiment 5

Larvae averaging 57.6 mm. total length, with hind legs just beginning to show differentiation into thigh and shank with rudiments of the toe points and a length of 3 mm., were transplanted with the thyroid glands of tadpoles of practically identi-

TABLE 3

DATE EXAMINED	ENGRAFTED ANIMALS		CONTROL ANIMALS	
	Average length	Hind legs	Average length	Hind legs
	mm.	*mm.*	*mm.*	*mm.*
July 14	57.6	3.0	57.3	3.0
July 22	58.0	3.7	57.8	3.0
July 28	58.0	5.7	58.0	3.5
August 4	58.0	6.9	58.5	3.5

cal size and developmental stage. The grafts were made July 14th.

Summarized briefly, the experiment shows that transplantation of the thyroid glands of tadpoles 57.6 mm. total length, hind legs 3 mm. into similar-sized animals, with legs of the same size, stimulated limb development approximately 4.4 mm. in twenty-one days. During the same time interval and under similar environmental conditions, it will be recalled that the implanted thyroid of a metamorphosing animal brings about nearly complete transformation. The average limb length of the engrafted animals was raised considerably by one exceptional individual of the culture which reacted to the implanted tissue by an extremely rapid growth of the limbs. This animal developed hind legs 12 mm. long in the same time it took the remainder of the culture to develop limbs 6 mm. long. The chief thing of interest in the experiment is the fact that tadpoles with hind legs 3 mm. long possess a thyroid mechanism beginning to show physiological activity and storage of the hormone as attested by the effect upon limb growth when transplanted. Morphological examination of such glands confirms the physiological findings as colloid is present, though some of the gland follicles are still very small.

Experiment 6

The thyroids of larvae averaging 62.5 mm. total length, with well-differentiated hind legs 6 mm. long, were transplanted into other animals of similar size and stage of development. The tadpoles were all full grown in regard to size, but backward in

leg development. It is somewhat doubtful if animals of this type would have transformed before winter if left unmolested in their normal habitat. The control larvae for this experiment passed the winter as tadpoles.

TABLE 4

DATE EXAMINED	ENGRAFTED ANIMALS		CONTROL ANIMALS	
	Average length	Hind legs	Average length	Hind legs
	mm.	*mm.*	*mm.*	*mm.*
July 17	62.5	6.0	62.5	6.0
July 24	62.6	7.2	63.5	6.3
July 30	63.3	11.6	63.6	6.3
August 6	63.3	13.2	63.6	6.8

The experiment shows that transplantation of the thyroids of full-grown larvae 62.5 mm. total length, with differentiated hind legs 6 mm., into similar larvae induces an increase in limb length of the engrafted tadpoles of approximately 6.5 mm. within twenty days. Within this same interval and under the same environmental conditions larvae with undifferentiated limbs but grafted with the thryoids of newly metamorphosed frogs practically complete metamorphosis. Along with increase in limb development of tadpoles goes increase in physiological activity of the thyroid glands. When the tadpoles are without differentiated limbs, transplantation of the thyroids of such individuals has little effect on limb development; conversely, when the limbs are beginning to differentiate and grow, transplants of the thyroid of such animals show physiological activity commensurate with the degree of limb development and differentiation of the larva from which it was taken. In all of these experiments it should be kept in mind that the standard of comparison used in determining the degree of physiological activity of various types of thyroids was the time interval required for the transplanted thyroid of a newly metamorphosed or metamorphosing frog, to practically completely transform an immature tadpole with no undifferentiated limb buds.

These experiments, by utilizing the method of gland transplantation, indicate clearly that thyroid conditions in anuran tadpoles differ markedly from those existing in the larvae of the caudate amphibia, such as the axolotl. In the latter, and the same is probably true of most urodele larvae, the thyroid manufactures and stores its physiologically active hormone, but apparently does not release it in sufficient quantities to induce metamorphosis until a certain degree of development is attained by the individual, when the hormone is suddenly released and the organism metamorphoses within a few days. In the axolotl the hormone may be stored indefinitely and metamorphosis never takes place. This is especially true of certain strains, notably that living in the vicinity of Mexico City, Mexico. In urodele larvae (axolotl) the onset of transformation is abrupt and the process generally completed within a short time. This point was clearly established by a recent series of transplantation experiments (Swingle, '22).

The evidence from the experiments recorded in this paper indicates that in anuran tadpoles the thyroid hormone is released into the blood-stream about as rapidly as formed, and only in neotenous individuals is the secretion retained within the gland vesicles to be abruptly released, somewhat after the fashion of urodeles. The process of limb development in anurans gives the clue to thyroid conditions in tadpoles because the rate of development of these structures is directly correlated with and dependent upon the amount of hormone manufactured and released by the thyroid gland.

This fundamental difference between urodele and anuran larvae in respect to retention in the one case and liberation in the other, throughout the period of larval life, of the thyroid hormone, appears to account for the differences in metamorphosis of the two groups. In urodele larvae transformation is initiated abruptly and quickly completed, whereas in anurans the process is gradual but cumulative, one body change following another, beginning with limb development in the early larva, and extending over a period of weeks or months, depending upon the species, until the completely metamorphosed frog results.

HETEROPLASTIC THYROID TRANSPLANTS

Experiment 7

The thyroid glands of Diemyctylus (Triton viridescens) are noteworthy because of their extremely rich blood supply; the difference between these salamanders and larval anurans in this respect is striking. Both thyroids of adult individuals were transplanted into single R. clamata tadpoles July 15th. The larvae used averaged 56.6 mm. total length with differentiated hind-limb buds 3 mm. just beginning to show differentiation of the toes.

Nine days following transplantation the larvae showed all the symptoms of hyperthyroidism, such as emaciation, protrusion of the eyes, growth of the limbs, and tail atrophy. The right fore leg was through the skin in five individuals on this date, in none had the left leg appeared. The frog mouth was very apparent in two specimens. July 25th three animals died; one had developed both fore legs. Tail atrophy was very marked in all three individuals. The cause of death appeared to be due to too rapid a rise in metabolic rate with resulting tissue disintegration. Complete autolysis of the skin of the pectoral region on the right side where later the fore leg appears was observed in every case to occur synchronously with or else slightly before the development of this organ. The most interesting thing about this experiment was the rapidity with which the metamorphic changes were initiated as a result of a heteroplastic graft of this nature where glandular tissue from such widely separated forms was employed (fig. 6).

The engrafted tadpoles of this culture developed much more rapidly than similar animals grafted with both glands of newly metamorphosed R. clamata frogs or with thyroids of animals in advance stages of transformation. That is to say, the heteroplastic transplant was far more effective in inducing metamorphic changes than the homoplastic grafts within the same length of time. The most probable cause for this is the quantitative difference in the amount of physiologically active hormone present in the two types of thyroid glands. In metamorphosing

animals or those that have just completed the process, more of the hormone is used up, possibly, in bringing about transformation than at any other time in the history of the individual; hence it is to be expected that the thyroids of such animals will be lower in physiological efficiency than those of adult forms either of their own or a different genus.

No special effort was made to ascertain the fate of the graft beyond the fact that it appeared to be resorbed something over two weeks after implantation.

It is possible to transform two larvae by successive transplantation of the same graft. At first thought it might seem rather startling that a thyroid transplant could be induced to metamorphose two immature tadpoles in succession, but in fact such a result is to be expected. The thyroid graft contained sufficient physiologically active secretion to metamorphose several such animals probably, and when implanted in tadpole no. 1 sufficient was absorbed to induce transformation. The graft was then extirpated and reimplanted in another animal, which in turn obtained sufficient secretion to induce metamorphosis. The transplanted tissue was undergoing resorption in both larvae. The writer recently practically metamorphosed five tadpoles with the thyroid of one axolotl, and very probably could have metamorphosed five more by extirpating the implanted tissue and reingrafting it in other animals. The thyroid tissue need not be viable to induce metamorphosis so long as it contains stored secretion, for this will be absorbed along with the glandular tissue and induce transformation as effectively as though released by a normal gland.

An excellent illustration of this was recently demonstrated by Mr. Carl Mason of this laboratory in the course of a series of thyroid-transplantation experiments. Mason extirpated the thyroid glands of a 14-inch axolotl and sliced them into ten pieces, each of which was then transplanted intraperitoneally into immature Rana sylvatica larvae without limbs. Three of the engrafted animals were thyroidless, the glands having been extirpated very early in embryonic life. All of the transplanted larvae promptly metmorphosed within ten to fourteen

days following implantation of the piece of axolotl gland. Not content with transforming ten larvae with one axolotl thyroid, Mr. Mason injected three others with the water in which the gland had been sliced to pieces. It is interesting to note that such an aqueous solution of thyroid substance was practically as efficient in inducing metamorphosis as the pieces of glandular tissue.

DISCUSSION

A. Is the prolonged larval existence of R. catesbeiana and R. clamata due to defective endocrine interrelation?

These experiments throw very little light upon the cause of the slow rate of thyroid differentiation in Rana catesbeiana and R. clamata. Animals such as those described in experiment 4 may possess glands more or less undifferentiated morphologically and practically inert physiologically in so far as the effect upon metamorphosis is concerned when they are transplanted. Why is it that such a great length of time is required by these animals for thyroid development? In most anurans the gland develops very rapidly and metamorphosis is completed within a single season, and in the case of toads, within a few weeks. The bullfrog larva does not require such a long span of larval life in order to fulfill its developmental requirements, because young animals can be induced to transform by thyroid feeding or transplantation.

When thyroid conditions in the short larval-lived frogs and toads are compared with those existing in the bullfrog and green frog, the evidence indicates that in the former group some factor is operating which functions as an 'activator' or stimulator of thyroid development and differentiation and also to release the physiologically active hormone when formed, for retention of the hormone within the gland vesicles appears to be common among the individuals of this group of anurans. In those forms with extreme prolongation of larval existence this activating factor is probably either inoperative or held in abeyance for long periods of time.

The question at once presents itself, What is the nature of this unknown thyroid-activating factor in anurans? Is it chemical (hormonal), nervous, or both? The question, though easily raised, is exceedingly difficult to answer definitely, since only a few of the facts in the case are even partially known. However, there is suggestive evidence from a series of transplantation experiments, that the unknown factor is probably a secretion liberated from the anterior lobe of the pituitary gland. This evidence on the interrelation of thyroid and pituitary, will be presented in detail, because of its bearing upon the causes probably responsible for the prolonged larval existence of the green frog and bullfrog and the problem of neoteny discussed later in this paper.

The clearest demonstrable cases of interrelationship known to exist between members of the endocrine system is that of the thyroid and pituitary glands. It is an odd fact that the functional correlation of these structures is best known in anurans. For years it has been known that extirpation of the thyroid leads to hypertrophy of the pituitary, but the reciprocal dependence of the thyroid for its normal development upon the presence of the hypophysis was first clearly shown by Adler ('14) who made this observation upon frog larvae from which he had previously removed the pituitary. Smith ('16) and Allen ('17) greatly extended Adler's work. These investigators found that removal of the hypophysis of frog embryos causes profound disturbances of growth and development. The larvae take on a silvery hue, grow slowly, and do not develop limbs or show any tendency to metamorphose even when kept for very long periods; that is to say, pituitaryless larvae become neotenous.

Examination of the thyroids of hypophysectomized animals shows that this gland remains in a rudimentary condition and is functionally inactive. The ablation of the hypophysis by preventing in some unknown way the normal development of the thyroid inhibits metamorphosis indefinitely. However, the induced neoteny, i.e., retention of the larval characters, is not merely due to the thyroid alone, because recent experiments indicate that the matter is not so simple. Smith ('20) was

unable to bring to complete metamorphosis a typical hypophysis-free tadpole by either thyroid- or thyroxin-iodine feeding. On the other hand, such treatment readily metamorphosed thyroidless larvae. Smith concluded that metamorphosis is dependent upon the active principle of at least two glands—the thyroid and pituitary; in the absence of either, the larval condition persists.

Allen ('20) obtained further evidence of the reciprocal interdependence of these two glands in producing metamorphosis. Allen transplanted the anterior lobe of the pituitary into thyroidless larvae and into hypophysectomized animals, the latter possessing vestigial thyroids. The engrafted pituitaryless larvae were markedly stimulated toward metamorphosis, the vestigial thyroid gland undergoing a pronounced development. Pituitary transplants into thyroidless tadpoles failed to evoke any metamorphic response, since no thyroid mechanism was present to respond to the stimulation.

These experiments show clearly that the thyroid gland depends for its normal development and differentiation upon the pituitary. The effect of the latter upon amphibian metamorphosis is through the intermediation of the thyroid which it activates, but both glands are apparently necessary for complete transformation. When the activating influence of the pituitary apparatus upon vestigial and physiologically undifferentiated thyroids is considered, we are led to the suspicion that the reason for the extremely slow thyroid development and differentiation, and consequent prolongation of larval life in such species as R. catesbeiana and R. clamata, is due to some defect of interrelation between thyroid and pituitary whereby the former is not stimulated sufficiently by the latter: 1) To develop to the stage requisite to induce metamorphosis in a single season; 2) to release the fully formed hormone in neotenous animals.

Bearing in mind the intimate physiological correlation shown to exist between thyroid and pituitary, we come now to an analysis of one of the most puzzling problems with which the student of amphibian metamorphosis has to deal.

B. Factors involved in anuran neoteny and metamorphosis

Neoteny (neos, young; teina, extend) means the extension of the youthful period, i.e., larval state, beyond the normal time required for metamorphosis. Kollmann ('82) distinguishes between partial neoteny, where the animal is simply retarded in metamorphosis beyond the normal time, and passes through the winter as a tadpole, and total neoteny, in which case the animal becomes sexually mature as a larva.

Partial neoteny is common, and there are numerous reports of anuran larvae which normally should have metamorphosed the same season they hatched, passing the winter as tadpoles and transforming the following summer. Such 'über wintern Larven,' as the German investigators call them, have been reported (as aberrations of the normal developmental cycle) for the following species: Bombinator pachypus; Alytes obstetricans, Hyla arborea, Rana esculenta, Rana temporaria, Bufo vulgaris, and Bufo viridis. These are all European forms, although in eastern North America we have some remarkable instances of partial neoteny. A great many tadpoles of Rana clamata remain extra seasons as larva, and the same is true of R. catesbeiana.

In cases of total neoteny, as already stated, the animal remains a larva and may become sexually mature as such. According to Kollmann ('82), this type of neoteny has been observed only in urodeles, and he cites the following species in which the phenomenon has been reported: Triton vulgaris; T. alpestris, T. cristatus, T. boscai, T. waltli, and Siridon, the axolotl larva of Amblystoma tigrinum. We are not concerned here with the neoteny of urodeles, as the writer has discussed this problem elsewhere in connection with experiments on axolotl, and the perennibranchiate, Necturus (Swingle, '21). However, total neoteny is not solely confined to urodeles, because there are several well-authenticated cases occurring in anurans. Thyroidectomized tadpoles kept for long periods after the normal period for metamorphosis readily ripen their sex products—at any rate in the male, according to Allen ('18) and Hoskins ('19). Sometimes the mature larvae of R. catesbeiana show ripe spermatozoa in the

testes (Swingle, '21). Recently, Dr. C. L. Parmenter, of the University of Pennsylvania, showed the writer sections of the testes of neotenous parthenogenetic tadpoles (Rana pipiens, obtained from Professor Loeb) which showed large numbers of spermatozoa. These facts clearly indicate that metamorphosis has nothing to do with germ-cell development and differentiation and that prolongation of the larval state in either urodeles or anurans leads to total neoteny if sufficient time is given.

Recently a giant R. catesbeiana larva 160 mm. long, hind legs 23 mm., was brought to the laboratory. The thyroid glands were not present, though evidently had been at one time, since the animal had hind legs 33 mm. The larva was sexually mature and had numerous sperm in the testes.

Attempts to explain the phenomena of anuran neoteny and its converse metamorphosis have been many and varied, but until 1912 the theories purporting to solve the problem were unsatisfactory, to say the least, and speculation ran rife. However, following the appearance of J. F. Gudernatsch's important paper detailing the results of feeding thyroid-gland substance to tadpoles, the question took on an entirely different aspect. Gudernatsch ('12), it will be recalled, fed immature larvae of Rana with fresh mammalian thyroid tissue and obtained a remarkable acceleration of metamorphosis. He was able to produce pigmy frogs scarcely larger than flies within a comparatively short time following thyroid administration. This experiment has been confirmed many times since (Morse, '14; Romeis, '13–'14, and Swingle, '18). This work indicated that anuran metamorphosis depends upon an internal secretion of the thyroid gland.

Allen and Hoskins, in 1916, working independently, succeeded in extirpating the thyroid gland of anurans at its first inception as an evagination from the pharyngeal floor, and hence at a time before the gland could possibly be physiologically active. They found that the operation prevents metamorphosis. Both of these investigators were able to keep their thyroidless larvae for long periods of time, and during this interval they attained a size far in excess of that normal to the species. Examination of the gonads of the giant larvae showed the presence of mature

spermatozoa. The operation of removing the thyroid apparatus had produced total neoteny in a species which never exhibits it under normal conditions. These experiments clearly indicate that metamorphosis and its reverse, neoteny, depend upon the thyroid.

Critical study of Gudernatsch's work on thyroid feeding led several investigators to the belief that in the last analysis anuran metamorphosis must depend upon certain factors in the food of tadpoles, which the thyroid gland extracts from the blood stream and elaborates into the thyroid hormone. Since mammalian thyroid is capable of inducing precocious metamorphosis, and as mammals have no larval period, it was naturally concluded that whatever the metamorphosis-producing agent might be, it must be common to the thyroid glands of all vertebrates. Iodine has long been known to be a constant constituent of the thyroid, and this element was seized upon as the possible cause of anuran transformation.

Morse ('14) fed larvae of Rana pipiens on various iodine compounds and succeeded in precociously transforming tadpoles by feeding iodized amino-acid (3-5-di-iodo-tyrosine) and iodized blood albumen. This experiment was later confirmed by Rogoff and Marine ('17), who were able to accelerate the metamorphosis of tadpoles by feeding artificially iodized ox-blood serum, the serum having no effect without the iodine. The globulin fraction of the serum contained most of the iodine. Similar results upon metamorphosis were obtained by feeding iodized casein.

The writer performed an extensive series of iodine feeding experiments on anuran larvae which gave some interesting results and which carry the work started by Gudersnatch one step farther. Normal and thyroidectomized frog larvae were fed inorganic iodine in very large quantities together with quantities of algae, and precocious metamorphosis resulted. It will be recalled that under normal conditions thyroidless tadpoles do not metamorphose, but remain permanently as larvae. The thyroid vesicles of the iodine-fed normal tadpoles showed a greater colloid content than those of the controls. Bromine had no effect upon metamorphosis when administered in equal

concentrations. Quantitative iodine-feeding experiments upon normal and thyroidless toad larvae where the animals were reared in iodine solutions of varying strength showed that larvae without the thyroid apparatus are unable to utilize extremely minute quantities of iodine absorbed through the integument, and do not metamorphose, whereas similar animals with intact thyroids readily respond to concentrations that have no effect upon thyroidectomized tadpoles. For instance, normal toad larvae with intact thyroids can be induced to metamorphose when reared in an iodine solution representing 0.0000003833 gram concentration within nine weeks when fed on an otherwise iodine-free diet. Thyroidless larvae of similar age reared in the same solution failed to metamorphose after sixteen weeks.[2]

The experiments indicate that iodine in some form or other is essential for amphibian metamorphosis and that the thyroid mechanism of these forms extracts from the blood stream the iodine taken in with the food and water, stores, and transforms it into the thyroid hormone by combination with other substances within the gland tissue. In weak concentrations thyroidless animals cannot utilize iodine. The question at once arises, how can thyroidless larvae metamorphose when fed iodine, since no glandular tissue is present? It will be recalled that Morse was able to accelerate metamorphosis by feeding iodized blood albumen. In this respect the iodized protein simulated the action of the thyroid hormone in so far as metamorphosis is concerned. It is the writer's belief that when thyroidless larvae are induced to metamorphose when fed elemental iodine in very large quantities, this substance combines in the blood stream with some amino acid or possibly the globulin of the blood, so that the end result is the same as if iodized blood proteins or amino-acids were fed, or else the iodine in solution is combined with substances in the protoplasm of the algae upon which the tadpoles feed. It is improbable that frog or toad larvae can use elemental iodine

[2] Such an extremely weak iodine concentration is barely sufficient to enable normal toad larvae with intact glands to metamorphose at all when fed on an otherwise iodin-free diet. Transformation is retarded.

per se as a metamorphosis-inducing agent, but only when this substance is organically combined in a certain way. Control animals fed on Spirogyra showed no metamorphic changes at the end of four months.

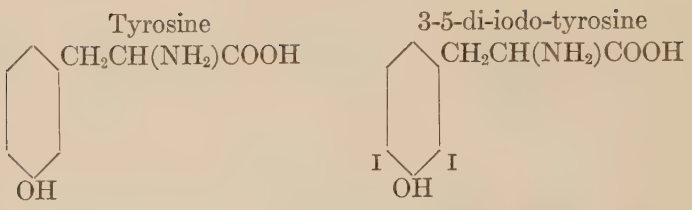
Allen ('19) fed inorganic iodine preparations to tadpoles whose thyroid and pituitary glands had both been removed in early embryonic life, and reported that his animals underwent a precocious and nearly complete metamorphosis on the iodine diet before death supervened.

Experiment 8

Recently the writer completed an experiment which shows very clearly the importance of iodine in inducing anuran metamorphosis, and indicates, moreover, that other halogens such as bromine cannot be substituted for it. A detailed account of this experiment is scheduled for publication elsewhere, hence only a very brief summary of the results will be included here.

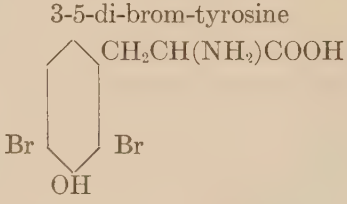
Thyroidectomized and hypophysectomized frog larvae (Rana sylvatica), the glands of which had been extirpated early in embryonic life, were kept until after the normal time for metamorphosis had elapsed and the normal controls had transformed, and then divided into three sets for experimental purposes. It will be recalled that thyroidless and pituitaryless larvae do not metamorphose, but remain permanent larvae unless fed thyroid derivatives or very large amounts of iodine. One set of animals was fed quantities of pure tyrosine each day and allowed to remain in weak solutions of this substance six to eight hours every day. The remainder of the time they were kept in large glass containers and fed large quantities of Spirogyra—an excellent food for tadpoles.

The second lot of animals was fed equal quantities of tyrosine in which two atoms of iodine had been substituted for two hydrogen atoms of the tyrosine, forming the well-known compound 3-5-di-iodo-tyrosine. The iodine in this compound is, of course, an integral part of the molecule.



For preparation of iodo-tyrosine, see Ostwald's method, vol. 59, p. 321, Zeitschrift für physiologische Chemie, and also the American Chemical Journal, vol. 33, p. 368, 1905.

The third set of tadpoles was fed quantities of tyrosine equal in amount to that received by the other two cultures, in which two atoms of bromine had been substituted for two hydrogen atoms of the tyrosine molecule forming the compound 3-5-di-brom-tyrosine.



The di-brom-tyrosine was prepared according to the method of C. T. Mörner, 1913, Zeitschrift für physiologische Chemie.

The results of feeding these tyrosine compounds to thyroidless and pituitaryless larvae is extremely interesting. The animals fed tyrosine and Spirogyra showed no changes indicative of metamorphosis after sixty days of continuous feeding, though the normal growth rate was not interrupted. After the twenty-fifth day the hind legs differentiated and grew very slowly until they attained a length of 3 to 5 mm. There were no signs of fore legs, skin autolysis in the pectoral region, tail shrinkage, or anything suggesting transformation.

The animals fed 3-5-di-brom-tyrosin behaved in identical fashion with those of the tyrosine-fed culture and no metamorphosis resulted. At the end of the second month of feeding the hind legs of the animals of the culture averaged 5 mm. The growth rate of the animals was not interfered with by the brom-

tyrosine compound. In so far as the acceleration of metamorphosis is concerned, the results obtained by feeding tyrosine and 3-5-di-brom-tyrosine are essentially negative.

Conversely, the administration of 3-5-di-iodo-tyrosine to thyroidless and pituitaryless larvae brought about very striking results, and in so far as metamorphosis is concerned, simulated the action of thyroid extract, although the effect of the latter is somewhat more rapid. Within a few days after feeding iodo-tyrosine the animals appear thin and emaciated; the limbs grow rapidly and the other changes incident to metamorphosis appear. Twenty days after the date of first feeding the entire culture of thyroidless animals had completed metamorphosis except for the loss of the tail. The pituitaryless animals developed fore and hind legs, frog mouths, but invariably died before tail resorption was complete. In two pituitaryless animals the right fore legs broke through the skin eight days after the animals were placed upon the iodo-tyrosine diet. These two individuals were exceptional in this respect, the average time being about twenty days. Figure 7 shows the effects of feeding 3-5-di-iodo-tyrosine to thyroidless and pituitaryless larvae.

These results are of significance for several reasons: 1. The experiment is clear-cut and admits of but one interpretation, i.e., that it is the iodine within the tyrosine molecule that is essential for anuran metamorphosis, because tyrosine without the two atoms of iodine is incapable of inducing metamorphosis.

2. Thyroidless and pituitaryless frog larvae do not metamorphose unless fed thyroid substance or very large quantities of elemental iodine. Such larvae have no thyroid mechanism for the manufacture of the thyroid hormone, yet apparently are able to utilize inorganic iodine when administered in very large quantities along with normal food. The substitution of the two iodine atoms for two hydrogens of the tyrosine molecule transforms the tyrosine into a highly active metamorphosis-inducing agent far superior to any quantity of elemental iodine in its rate of action and second only to the thyroid hormone itself. It seems clear that in tadpoles metamorphosis depends upon an organic iodine complex of some sort, and that the

iodine to be effective does not need to undergo transformation within the thyroid gland. It is evident that when thyroidless larvae metamorphose when fed large quantities of inorganic iodine, the latter to be active must enter into organic combination with either the body proteins of the larvae or the algae fed with it.

3. Iodized proteins and amino-acids have been employed by several investigators in the treatment of various disorders of the thyroid in cases of human hypothyroidism, but the writer has never heard that such products can completely take the place of the thyroid hormone. Yet in thyroidless anuran tadpoles iodo-tyrosine to all intents and purposes is as good as the thyroid secretion in transforming the individual, i.e., it completely takes the place of the gland secretion in so far as metamorphosis is concerned.

Metamorphosis in anurans is dependent upon a peculiar property of the iodine atom when organically combined in a certain way, and it seems that mammals and anurans are not to be compared in regard to their reactions to iodine. Thyroidless tadpoles promptly metamorphose when fed iodized amino-acids or large quantities of elemental iodine, but thyroidless mammals and individuals with atrophied and degenerate glands cannot utilize elemental iodine at all, and the same is probably true of iodized amino-acids. Voegthlin and Strouse ('09) (Journal Pharm. and Exp. Therap.) observed that iodized amino-acid fails to replace the thyroid function in pathological cases of hypothyroidism, i.e., myxedematous and cretinous mammals, nor was the nitrogen metabolism or blood pressure of dogs influenced by administration of this substance. Furthermore, Miura ('22) had made the interesting observation that di-iodo-tyrosine gives no protection to mice against acetonitrile, whereas thyroid tissue protects these animals against lethal doses of the poison.

It is clear that, in so far as the metamorphosis of thyroidless and pituitaryless tadpoles is concerned, iodo-tyrosine is practically as good as the thyroid hormone, but this same substance in mammals cannot be substituted for the glandular tissue itself.

4. The suggestion of some recent writers that bromine if it could be substituted for the iodine of the thyroid might prove just as active physiologically, is not borne out by these experiments. Bromine has no influence upon anuran transformation

Fig. 7 The effect of feeding iodized tyrosine to thyroidless and pituitaryless R. sylvatica tadpoles. *A*, thyroidless larvae fed iodotyrosine fifteen days; *B*, thyroidless control fed tyrosine forty-two days; *C*, pituitaryless tadpole fed iodo-tyrosine sixteen days; *D*, pituitaryless control fed tyrosine forty-two days.

and cannot be substituted for iodine. It is merely begging the question to say that tadpoles have no glandular mechanism for the utilization of bromine, hence their inability to react when subjected to its influence, because in our experiments thyroidless larvae were used, forms which have no mechanism for utilizing iodo-tyrosine either, yet the response to the latter is very prompt.

So far as known at present, iodine in some form or other is the only agent known aside from preparations of thyroid-gland substance (which contains iodine as an integral part of its physiologically active hormone) capable of inducing metamorphosis of thyroidectomized larvae and very advanced stages of transformation in hypophysectomized animals.

Hoskins and Hoskins ('20) reported precocious metamorphosis of normal and thyroidless tadpoles fed desiccated anterior-lobe pituitary substance. Smith ('21) subjected their results to critical analysis and finds that the pituitary tissue used by them was unique in its high iodine (thyroxin iodine) content, and that the metamorphosis was due to the iodine present and not to any peculiar property of anterior-lobe pituitary tissue to accelerate metamorphosis when fed.

Further evidence that iodine in some form is necessary for anuran metamorphosis has been furnished by Emmett and F. P. Allen ('20). In previous work ('19) these investigators had found that frog larvae grew readily enough when fed on various diets of which every constituent was known, including the accessory food factors (vitamines), but would not undergo metamorphosis. They concluded from their earlier work that some unknown substance was necessary to enable the animals to transform. Later ('20) they stated that adding iodine, either inorganic or organic, to their diets enabled the tadpoles to complete metamorphosis. Apparently their special diets were iodine-free, or at any rate contained but very minute amounts of this halogen. It is interesting to note that retention of the larval character of these animals, after the normal time for metamorphosis had passed, is a case of artificially induced neoteny due to lack of iodine in the diet. Though Emmett and Allen do not stress this point in their work, nevertheless the fact remains.

Abelin ('20) reports that tyramine accelerates the metamorphosis of tadpoles, and this is very markedly increased by putting iodine into the molecule—Dijodtyramin.

Jensen ('20) reports that feeding inorganic and several organic preparations of iodine to axolotls has no effect upon metamorphosis, but that thyroid extract readily metamorphoses

these neotenous forms. One organic iodine compound, iodothyrosin, induced transformation when fed, but was not so effective as the thyroid substance itself. Jensen suggests that axolotls be used to test the purity of thyroid preparations, since they react so readily to thyroid administration. However, since iodine entering the organism is picked up by the thyroid gland and elaborated into the thyroid hormone, and since in axolotls the thyroid is apparently unable to secrete its fully formed products into the blood, it is obvious that axolotl cannot metamorphose when fed iodine, and the same is true of neotenous anurans (Swingle, '21). The effect of thyroid feeding upon axolotl metamorphosis appears suspiciously like a stimulation effect upon the organism (in this particular case) whereby the thyroid inhibition is overcome and the hormone released. There are too many other agents that stimulate axolotl metamorphosis for any of them to be specific, except in so far as they enable the animal to overcome the thyroid inhibition. In a later paper Jensen ('21) found that axolotls may be induced to transform by intraperitoneal injections of iodocasein, iodoserumglobulin, and iodoserumalbumin.

Uhlenhuth's ('21) claim that iodine has no effect upon urodele transformation cannot be considered here, because this writer appears to labor under the delusion that all iodine is inorganic. Despite the fact he insists that iodine has no effect upon urodele metamorphosis, he used iodothyrine as a control substance and states that it will transform salamander larvae in thirteen days. Iodothyrine, at any rate, as isolated by Baumann, is not the active principle of the thyroid gland and has been so regarded by physiological chemists for many years. In fact, this substance does not exist as such within the thyroid and is produced artificially by the brutal action of sulphuric acid on the thyroid and is a result of decomposition of iodized substances within the gland. Iodothyrine as isolated by Baumann ('95) is an iodine compound containing a large per cent of iodine together with peptids. Kendall's thyroxin which contains 65 per cent of iodine as an integral part of the molecule must also be regarded as an iodine compound. This last substance has all the effects of thyroid

tissue when fed to either urodele or anuran larvae. In all of the substances so far used for inducing amphibian metamorphosis iodine is a constituent part. Elemental iodine, as such, however, appears to have no such effect upon the caudate amphibia as upon anurans as the former are unable to utilize it to the same extent.

A propos of Uhlenhuth's claim that iodine has no influence upon axolotl metamorphosis, it will be recalled that Jensen ('20) was able to induce axolotl to transform by feeding an organic iodine compound and by injecting iodized proteins such as iodocasein, iodoserumglobulin, and iodoserumalbumin. According to Kopéc (Biol. Bull., vol. 52, 1922) Hirschler ascertained that metamorphosis is accelerated in axolotls by injections of iodoform or iodine. The following reference is given by Kopéc: J. Hirschler, '18–'19. Sur la métamorphose provoquée chez l'axolotle à l'aide d'iode et des expérience apparentées. Extrait de Kosmos, Bull. d. L. Soc. polonaise D. Naturalistes à Leopol. The writer has been unable to obtain this paper.

The iodine required by amphibian larvae is of course obtained from the food and water and built up into the thyroid hormone by the addition of other substances within the gland vesicles or in the absence of glandular tissue as in thyroidless tadpoles, by other tissues. The wide distribution of iodine in plant and animal tissues is surprising, in fact, few food-stuffs and surface waters have been found which do not show distinct traces upon analysis. Sea-water, all marine animals and plants contain iodine, the same is true of the blood and tissues of all vertebrates. Iodine has been reported present in the following plants: sulphur bacteria, diatoms, blue, green and brown algae (various species) mushrooms, lichens, garlic, onions, leek, sorrel, red beet, beet, spinach, radish, turnip, peas, beans, egg plant, tomato, cucumber, pumpkins various kinds of melons, lettuce, cabbage, asparagus, and prairie hay. Cameron ('14, '15) gives an excellent review of the literature on this subject.

The various experiments on iodine feeding showing the necessity of this substance in some form or other for anuran metamorphosis lead naturally to the assumption that in districts where neoteny is prevalent the environment is possibly deficient in

iodine, or else that the thyroid glands of neotenous individuals are for some reason unable to utilize the iodine present and transform it into the physiologically active hormone. In a former paper ('20) the writer merely mentioned such possibilities as being suggestive, but investigation has shown the untenability of these possibilities. The writer ('22) has clearly demonstrated that the thyroid glands of axolotl are fully developed and filled with the physiologically active hormone, but the gland is apparently unable to excrete its products into the blood stream because of some inhibiting factor. Furthermore, search through the literature has shown that iodine is found in the vegetation making up the floating gardens of fresh-water lakes near Mexico City, the home of axolotl (Cameron, '14–'15, p. 353). Oversized neotenous anuran larvae with differentiated hind legs 11 to 12 mm. long, remaining extra seasons as tadpoles have active thyroid mechanisms, as shown by transplantation experiments, hence the iodine content of food and water have little if anything to do with the non-metamorphosis of neotenous individuals.

Here, too, just as in axolotl, the evidence points to an inhibiting factor which prevents the thyroids of such animals from secreting their fully formed hormone into the blood stream. According to writer's view (Swingle, '22), *neoteny both in urodeles and anurans is due to inhibition of the secretory (excretory) functions of the thyroid gland rather than to any morphological defect of glandular structure, or inability to synthesize the physiologically active hormone.*

Adler ('16) has made an interesting attempt to bridge the gulf between the older exogenous and newer endogenous theories of the causation of neoteny by studying the effects of extremes of temperature on the development of Rana temporaria and on the structure of the thyroid gland. The type of thyroid in larval and adult frogs varies according to the climate. It is large and made up of many small follicles in Alpine frogs, i.e., those from cold regions; and is small and possesses relatively few large follicles in the Adriatic frog or those from warm regions; in temperate regions such as Germany, the thyroid is of an intermediate type. When larvae were subjected to extremes of temperature, growth and metamorphosis were delayed. Since microscopic examina-

tion of the thyroid showed atrophic changes, Adler concluded that extremes of temperature play a causative rôle in neoteny by initiating regressive changes in the thyroid, thus correlating the older theory of extrinsic environmental factors with the newer endocrin view of the causation of the phenomenon in amphibians.

Adler's view is interesting but does not explain amphibian neoteny. In the first place, as shown by the writer ('21), axolotls have a normal thyroid gland and normal secretion, the same is apparently true of neotenous anurans; only the secretion cannot escape into the circulation unless the gland is extirpated and transplanted, apparently because of the inhibition of some agent in the neotenous individual which normally serves to release the thyroid hormone into the blood stream. On the other hand, environmental factors, such as extremes of temperature, may have considerable to do with developing the inhibiting factor or factors responsible for the failure of the thyroid to release its secretion at the proper time. The nature of the inhibiting factor or factors is obscure, but appears to be due in part at least to some defect of that portion of the individual's nervous system responsible for the secretory (excretory) functions of the thyroid gland. However, any discussion of neoteny must take into consideration the intimate functional correlation existing between the various components of the endocrine system, especially the thyroid and pituitary. Some defect of interrelation between these two glands seems likely in fully developed cases of neoteny, although more experimental factors undoubtedly enter into the chain of causes responsible for neoteny, but as nothing is known concerning them, discussion is futile.

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THE EFFECT OF EXTIRPATION OF THE EMBRYONIC EAR AND EYE ON EQUILIBRATION IN AMBLYSTOMA PUNCTATUM

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The observations here reported on the equilibrium of Amblystoma grew out of an investigation started by one of us (Laurens) in the spring of 1919 at the request of Prof. R. G. Harrison. Doctor Harrison wished information concerning the development of functional activity for correlation with the structure of ears of larvae following transplantation and rotation of the ear vesicle in early stages of development. In order to obtain this physiological knowledge of the probable structure of the ear vesicle, the locomotion, reaction to rotation, etc., of normal Amblystoma, as well as of those from which one or both ears had been removed was investigated. In addition to studying the effects of removing the ear, the results of removing the eye, on posture, at rest and during locomotion, as well as on rotatory compensatory movement were observed.[1]

All possible combinations of earless and eyeless larvae were obtained by extirpating the rudiments of these sense organs from early embryos (stages 27 to 29, Harrison). Observations were made at frequent

[1] These eye observations were made by Greene, who also separately repeated some of the observations on the ear in connection with another problem.

The material used for study is taken from two different seasons and two separate operators (Harrison and Greene). The first animals were operated by Harrison and turned over to Laurens for study. The results obtained led the latter to suggest that a complete series of all possible eyeless and earless types be made. Although not all types of operations were represented in each of the four series, the 1921 series (Greene) was complete and representatives of every type were successfully reared through metamorphosis. These were demonstrated at the Christmas meeting of the Anatomists at Yale University, 1921. During the following spring additional operations were made in order to repeat certain observations, as well as to fill in a series of stages, sections of which are now being studied.

intervals, until some time after metamorphosis (6 months in many cases) for disturbances in equilibrium with the animals at rest, while crawling, swimming, alighting after slow and quick swimming, during and after horizontal rotation, and during and after tilting of the horizontal axis.

No review of the literature dealing with the various phases of the functions of the parts of the labyrinth in equilibrium will be undertaken. The authors referred to are those whose work is most intimately connected with the specific problem of this communication. So far as we know the paper of Laudenbach (1) is the only work on a Urodele. Laudenbach removed the labyrinths (one or both) from larval and adult Siredon pisciformis. The typical results of swerving, rolling, etc., to the operated side following one-sided removal were obtained, being particularly striking in young individuals (6 to 10 months). In older animals (over a year) abnormal locomotion disappeared in 3 to 4 days after the operation, to reappear only upon stimulation. Laudenbach regards this disappearance of abnormal posture as due to a functional compensation on the part of the remaining labyrinth, since he obtained, upon later removing this, the typical phenomena of a double labyrinth extirpation.

An extreme incoördination was found by Laudenbach to follow removal of both ears, the animals swimming in circles, deviating haphazardly in all directions, and finally sinking exhausted to the bottom, coming to rest most often on their sides, but also upon their backs. Upon recovering from fatigue, a normal position was assumed. Such animals usually remain at rest unless stimulated. When they rise to the surface for air, they reach there only with the greatest difficulty. Their position at the surface is abnormal and conditioned by the specific gravity of the body. On the bottom they orient fairly well, due to the assistance of the tactile sense in the extremities. Crawling is awkward. Removing in addition the four limbs produced a complete incoördination. Obtaining (snapping for) and swallowing of food is markedly affected, and the food has to be pushed down into the esophagus.

Laudenbach investigated the question of the importance of the otoliths in causing the abnormalities just reviewed. He removed the otoliths, leaving the semicircular canals intact, and found that the operation did not result in any disturbance of equilibrium or of locomotion. He concluded, since the removal of the entire labyrinth results in incoördination, while that of the otoliths alone does not, that the

otoliths have nothing to do with equilibrium. Possibly, if Laudenbach had removed the semicircular canals alone, he might have obtained somewhat similar results. (See Maxwell (2).) Laudenbach, however, obtained similar results on the frog and states that without otoliths this animal is practically normal in its locomotion and gives typical reactions to rotation and tilting.

Results. From observations on normal larvae it was ascertained that the ear begins to function as an equilibratory organ at stage 40 to 41 (length 12 to 14 mm.). At this stage the balancers are completely formed, the eyes are movable, the gills well branched, and the forelimbs just starting to bifurcate, although not yet functional. The swimming of the larvae is to all intents perfect and the animals are quite active, although the yolk persists for 5 to 7 days more, and it is generally 7 to 9 days before the mouth breaks through.

Streeter (3) determined that the equilibratory function begins in frog tadpoles (R. sylvatica) at the sixth day of development, sections showing that the labyrinth then consists of a closed epithelial sac incompletely divided into compartments and possessing differentiated nerve endings which are connected with the brain. Herter (4) believes that the results following upon the removal of the labyrinth (in six species of Anurans) can be detected at an earlier stage than this in so far as locomotion is concerned. He observed, when tadpoles were rotated in water, that they gave characteristic reactions such as to cause them to swim in the direction opposite to that of the rotation. He gives no information concerning the stage when this reaction was first seen. He did, however, determine that the post-rotatory reaction was first elicitable at that state of development when the horizontal semicircular canals have been separated off, although only a part of the canal is in the form of a tube.

Our notes show that in Amblystoma the post-rotatory reaction (described below) is not obtainable in larvae less than 20 mm. long, but is in those 23 mm. long.

No determination was made on the larvae here studied as to the probable time when the eyes begin to function. Laurens (5) in a series of observations on the determination of the stage of development at which the melanophores first respond to light and darkness, demonstrated that the secondary responses of the melanophores, which are dependent upon the eyes, are first exhibited by Amblystoma larvae when they are between 17.7 and 18.4 mm. long, and that the larvae begin to feed when they are between 16.7 and 18 mm. long. Detwiler

(unpublished results) has also demonstrated that the future cones in the retina of Amblystoma at stage 44, at which time the characteristic form of the rods has not yet been developed (see Detwiler and Laurens (6)), shorten in light and lengthen in the dark.

Observations on normal Amblystoma: *a. Resting posture.* As above recorded, the labyrinth exerts no observable influence over equilibrium until stage 40 to 41 is reached. Before this period, although the animals have been free-swimming for some time (since stage 36), they come to rest after swimming in rather haphazard positions, that is to say, they may fall to the bottom on their sides, or backs, as well as come to rest on the ventral surface. This, however, is no longer the case after stage 40 to 41 has been reached. The animals now drop to the bottom symmetrically, poised on an even keel, and show the typical posture, with limbs symmetrically placed.

b. Reactions to horizontal rotation and tilting. It is unnecessary to describe in detail the responses of normal Amblystoma to rotation in a horizontal plane around the dorso-ventral axis, since these reactions are similar to those of other vertebrates which have been described so often. Briefly, when an Amblystoma is rotated on a horizontal turn-table, it assumes a typical compensatory position with the head bent (at the neck) in the direction opposite to that of the rotation, with the arm on the side to which the head is bent sharply flexed at the elbow and adducted, while the other arm is sharply extended and abducted. The body and tail of the animal are also correspondingly curved to the side opposite that of the rotation. If the rotation is continued for some time, and at an appropriate rate of speed, the animal, at intervals, quickly jerks its head in the direction of the rotation back to approximately the normal position, at which time the arms are placed symmetrically, after which the animal again slowly assumes the compensatory position.

When the turn-table is stopped rather suddenly, that is, not too slowly, the Amblystoma performs a typical post-rotatory compensatory movement. The head is slowly but decisively moved in the direction of the rotation, beyond the median line, with a slight flexion and adduction of the forelimb on this side, and a slight extension and abduction of that on the other. This position, which may be kept for some little time, gradually gives way to the normal.

In some cases the animal performs, during rotation, locomotor movements in a circle in the direction opposite to that of the rotation, followed by the post-rotatory reaction above described when rotation

is stopped. Again, however, the animal, when the rotation is very suddenly stopped, may actually make a locomotor movement in the direction of the former rotation. These reactions are given by larvae of all stages (after labyrinth function begins) and by adults.

The larvae were, when rotated, placed in a small dish containing a minimum of water. This was to obviate the possibility of the water currents set up acting as stimuli. This was further controlled at various times by anesthetizing larvae in chloretone and then rotating them. The passive position assumed under these conditions can not be confused with the compensatory positions. The adults were placed in a dry dish when rotated. The animals were as nearly as possible placed in the middle (over the center) of the dish. It is worth while recalling that the relative position of the animal with respect to the axis of rotation, that is, as to whether it faces toward or away from this (7), or is even placed tangentially on the turn-table (8), does not influence the direction of turning. The animal turns during the rotation in the direction opposite to that of rotation, and after rotation in the direction of the rotation. Herter (4) records that when tadpoles are placed on their backs they give the post-rotatory reaction just as they do when they are resting on the ventral surface.

In order to see the movements of the eyes during and after rotation, the animals were observed under a binocular while being slowly rotated. To produce these eye movements in Amblystoma, the turn-table must be rotated very slowly so that movements of the head are not produced. If the rate of rotation is too fast the eye simply deviates during the rotation. Ivy (9) has recently reported these rotatory and post-rotatory movements for the frog. They are not always obtained in Amblystoma, but are present in a large percentage of animals. In general it may be said that as the rotation is continued the deviation of the eye in the direction opposite to the rotation increases up to a certain point, at which the quick component intervenes, and the eye is jerked back, apparently not quite to its normal position, and the process is repeated as long as the rotation is continued. A typical post-rotatory nystagmus (that is, a slow deviation in the direction of the former rotation, with a quick return to the normal position) was not, it is believed, ever seen. Certain post-rotatory eye movements were observed, and these will be recorded. In some cases when the turn-table is suddenly stopped, the eyes are observed to be in the characteristically deviated position, which position is retained for a few seconds and then the eye is quickly jerked back to the normal position.

In others, when a more rapid rotation is stopped, the eyes are immediately snapped back to the normal position. Again, just after a slow rotation is stopped, the eyes are jerked in the direction of the rotation, but beyond the normal position, from which they are slowly returned to the normal; or they may be simply jerked back to the normal position. The eye movements of adults are similar to those of larvae. These differences in the post-rotatory eye movements are due to the suddenness with which the rotation is stopped.

The attempt to separate the head and eye deviations of Amblystoma caused by labyrinthine stimulation from those due to the stimulation of moving retinal images was not always successful. Loeb (10) found by surrounding the lizard Phrynosoma with an opaque cylinder, or by closing the eyes, that the compensatory movements of the head during rotation can be considerably diminished. Further, if the eyes of the lizard are closed and the animal rotated very slowly, the rotatory compensatory motions of the head and body are very slight, while the post-rotatory compensatory motions are powerful. When the eyes are open the reverse is obtained,—strong rotatory motions and slight post-rotatory. In some cases this is also found to be the case in Amblystoma, when the dish containing the animal is placed in another with high opaque walls. The post-rotatory movements of the head and eyes are very marked, being far beyond the median line, with a slow return to the normal position, while the movements during rotation (deviation and quick return) are very slight.

The slow deviation of the eyes of Amblystoma does not seem to be markedly influenced by moving retinal images, but to be chiefly controlled by the labyrinths. When the animal is surrounded by an opaque wall and rotated slowly, the eye movements seem to be, on the whole, similar to those above described. In some cases, however, there were no movements of the eyes to be seen during rotation, but when the rotation was stopped there was a sharp jerk of the eyes in the direction of the rotation.

The adults show a very typical reaction when they are slowly inclined from the horizontal. When the dish containing them is so tilted as to raise slowly the anterior end of the animal it responds by lowering the head until, as the tilting continues, it touches the bottom, raising the elbows (flexion) and finally climbing up the inclined plane. If the posterior end of the animal is raised, the reaction consists in a raising of the head, an extension of the arms until the animal either backs up the inclined plane, or turns around and climbs up.

When the container is tilted sideways, the animal reacts by rotating the head as if in an attempt to keep the horizon level (the eye movements were not studied) while the limbs on the upper side are flexed and those on the lower side extended. At intervals the lower extended forelimb executes "pawing" movements (of alternating extension and flexion) as if seeking a higher support.

Observations on animals deprived of one ear vesicle: *a. Resting position.* Some of these animals may at times, particularly at rest, appear perfectly normal with no apparent head deviation and torsion, and no asymmetry in limb posture. Nevertheless, the posture of such animals is throughout life a clear diagnostic indication as to which labyrinth has been removed. The head is bent at the neck toward the operated side, and exhibits a torsion toward this side. The arm on the operated side is flexed at the elbow, while the other is extended. The body is thus tilted in the same manner as the head (clearly indicated by observing the dorso-caudal fin) and shows a pleurothotonus with the concavity on the operated side. This abnormal posture complex is most obvious just after locomotion (swimming) has ceased. By drawing the animal up into a large pipette and gently releasing it into the water, it may be seen that as the animal sinks the body is inclined to the operated side with a flexion of the forelimb on

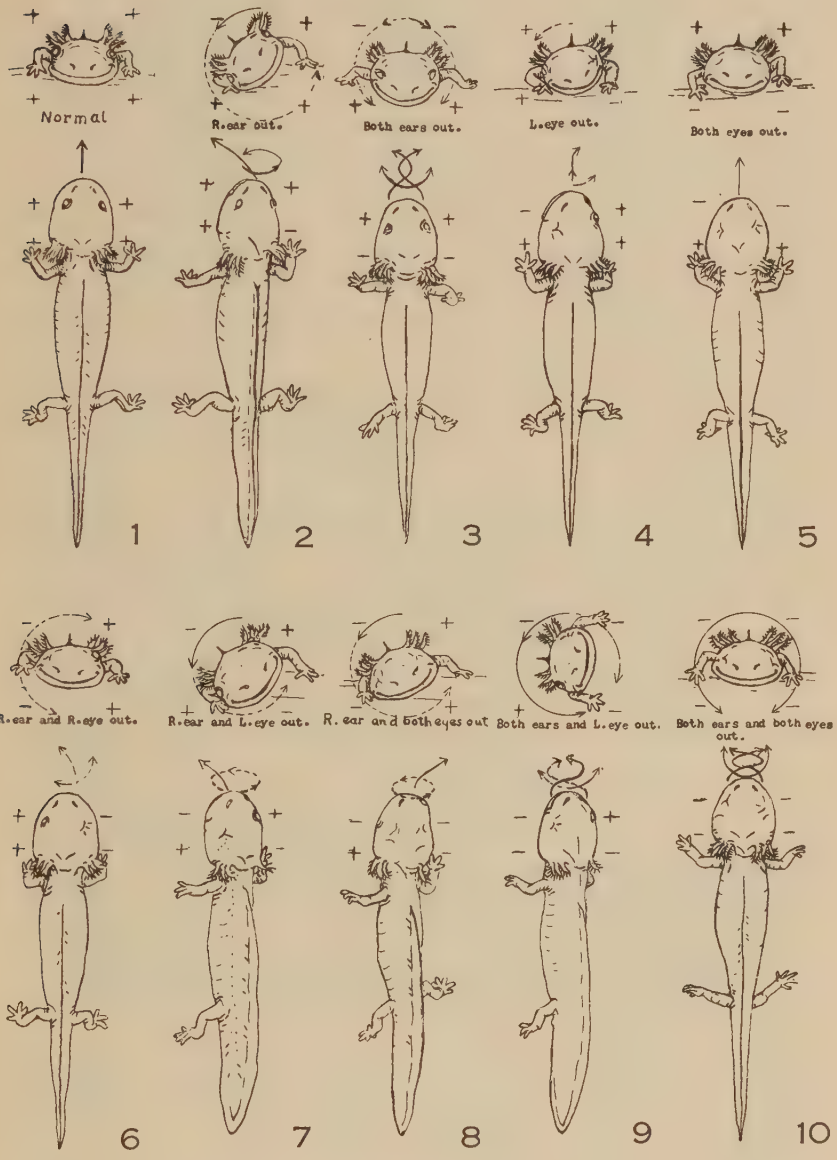
Plate I. Somewhat conventional views of each type of larva at approximately 80 days of age. The upper figure of each pair represents the anterior view of the operated specimen in its most normal resting posture.

The plus and minus signs above and to each side of the head indicate whether each respective ear is present or absent.

The same signs below the head record the presence or absence of the eyes.

Continuous arced arrows denote the direction of list during rest or crawling; while the dotted arcs, when present, denote the direction and amount of rolling to which the animal is commonly subject during swimming or lunging motions. Both arcs are indicative of the abnormal posture of the animal in relation to vertical and horizontal planes passing through the long axis of the head, and the demarcation of the dorsal portion of the caudal fin indicates whether or not the body list is equivalent to that of the head.

The lower figure of each pair shows the same type as seen from above, to illustrate more clearly the posture of the entire body at rest, after crawling or swimming. Here the plus and minus signs are placed near the eyes and ears to indicate their respective presence or absence; and the arrows at the anterior end of the animal show the deviation from a straight normal path during locomotion. The supplementary arced lines indicate here, as in the above diagram, the list or roll of the animal on its long axis during locomotion. Where such lines are continuous they represent the ruling tendency, while dotted lines indicate that the denoted deviation is commonly recurrent but not always involved.


Normal
R.ear out.
Both ears out.
L.eye out.
Both eyes out.
1
2
3
4
5
R.ear and R.eye out.
R.ear and L.eye out.
R. ear and both eyes out
Both ears and L.eye out.
Both ears and both eyes out.
6
7
8
9
10

that side and extension on the other. (Plate I, fig. 2; plate II, figs. 2, 3, 4, A, B, C, D, E, F, G; plate III, fig. 11A.)

b. Locomotion. Just as in posture, the locomotion of these animals may, at times, be apparently normal, yet for the most part the manner of locomotion is indicative of a disturbed equilibrium and is a diagnostic index of the side of the lesion. The animals usually swim on the operated side with emprosthotonus (or slightly tilted to this side) and either swerve slightly toward the normal side, or circle about the normal side as a center. They may, however, also roll over to the operated side on their backs when swimming and thus continue rotating on the long axis and swimming to the good side. The latter is particularly characteristic of vigorous locomotion, whether spontaneous, or the result of tactile stimulation. To quote from notes on one observation (AV62), "At rest apparently normal; swims on right side, swerving constantly to left; as comes to rest shows marked twisting and bending of head to right" (the right ear vesicle had been removed). Again (AV71) "At rest apparently normal; spontaneous locomotion seems normal, but when stimulated turns over on right side, swerves to the left; if swimming is very rapid, circles to the left." A rather marked opisthotonus sometimes causes these animals to swerve toward, or to circle about, the operated side.

If an animal is observed during a swim upward to the surface for air, it is seen, as it comes up on the slant, to roll over to the operated side, to swerve to the normal side and to rotate around the longitudinal

Plate II. Photographs of living animals in typical resting postures.

Where not specifically mentioned the photographs are each taken from a focus perpendicular to the horizontal plane upon which the animal is poised, after immediately precedent locomotion (crawling or swimming).

Fig. 1. Normal adult of about 9 months.

Figs. 2 and 3. Larvae of eighty days from which the left and right ears respectively have been taken in the tail-bud stage.

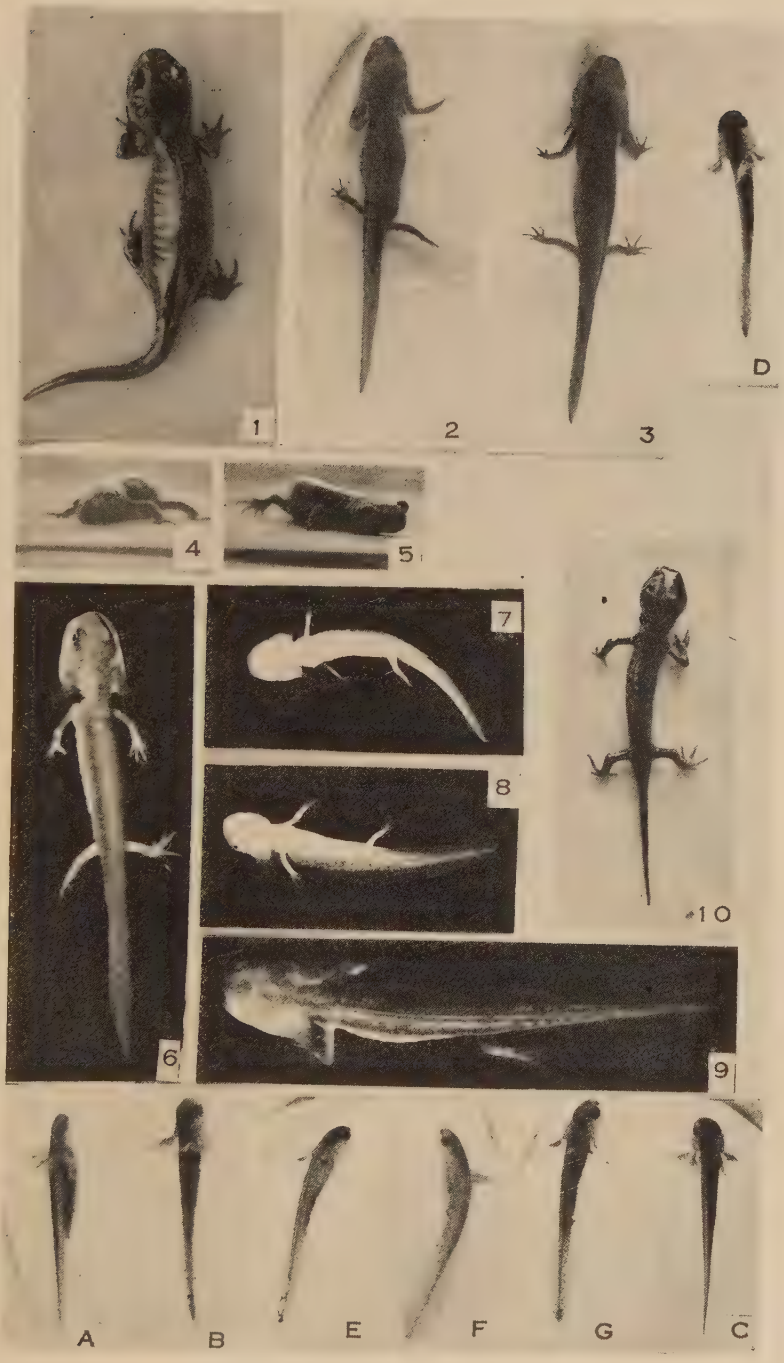
Fig. 4. Adult of eleven months, lacking its right ear. Anterior view.

Fig. 5. Normal adult of same age as figure 4. Anterior view.

Figs. A, B, C, D, E, F and G are various earlier larval stages of the single-ear extirpation type showing the variety of abnormal postures of rest assumed. That picture labelled *A* in the series is approximately the embryonic stage at which balance is definitely acquired. Picture *F* is a case where the right ear is lacking, while all the rest were operated upon the left side.

Figs. 6, 7 and 8 are of the type lacking both ears, and represent common postures assumed.

Figs. 9 and 10 are of the type lacking both eyes; 9 is a 90-day larva, while 10 is a young adult.


1
2
3
D
4
5
6
7
8
9
10
A
B
E
F
G
C

axis to the operated side until it reaches the surface, which it may do on its back. Having taken a gulp of air it slowly sinks, exhibiting the abnormal deviation described above which becomes more pronounced as it strikes bottom. Larvae, when crawling along the bottom, and adults exhibit the tilting (deviation) and swerving toward the operated side.

c. Reactions to horizontal rotation and tilting. The compensatory movements during rotation and immediately after its cessation are typically different from those exhibited by normal Amblystoma. When the direction of rotation is toward the operated side, the compensatory movement of head, limbs and body is slight. It may, in certain cases, not carry the posture back to the normal and hardly ever carries it to the side opposite the direction of rotation. In many cases there is no compensatory movement observable. In those cases where a rotatory compensatory movement of deviation is present, the quick jerk back in the direction of rotation and beyond the median line is very decided, the head (with the characteristic limb position) being held deviated in this direction for some seconds. The animals, under these conditions, often make circular movements drawing themselves over by the flexed arm. The post-rotatory movement is also very decided. The reverse is true when the direction of rotation is toward the normal side; the rotatory compensatory movements are very extensive, the head and body being deviated and bent far over to the side opposite that of the direction of rotation (the side lacking the labyrinth), with the corresponding limbs flexed (adducted) and extended (abducted) respectively. But the quick component of the compensatory reaction is hardly ever present, and when observed is but slight. The post-rotatory movement is weak, and often absent, the deviation to the side of the rotation (the side lacking the labyrinth) persisting for some time, until the animal moves, either spontaneously or as a result of mechanical stimulation, when the deviation may be decreased. It is in the cases where the resting compensatory posture (deviation) is particularly marked that the compensatory motion during rotation toward the side lacking the labyrinth is noticeably absent. The same is true of the post-rotatory motion when the direction of rotation is toward the side possessing the labyrinth.

When such an Amblystoma is rotated in a dish with opaque walls so that the excitation due to moving retinal images is removed, the differentiation between visual and labyrinthine stimulation is particularly clear, as it is superimposed on the effects due to the absence of

one labyrinth. When the direction of rotation is toward the operated side, there is but slight, in most cases no, compensatory movement toward the side opposite the direction of the rotation, the head, etc., often being kept deviated to this side, that is, toward the side without the labyrinth. The post-rotatory reactions, however, are most striking, the deviation, tilting, etc., to the operated side (the direction of the rotation) being very great, so that the animal actually falls to this side. When the animal is rotated toward the normal side, there is a decided compensatory movement directed to the opposite side. It is, however, not so great as when the opaque walls are not present. The post-rotatory compensation was hardly ever observed, and was but slight, when it occurred.

The ocular responses to rotation are in general similar to those observed for normal animals. Each labyrinth influences the eyeball rotations of both eyes, and when one is removed there is still binocular movement. But there is evidence of a differential reduction in tone of the ocular muscles, for in some cases when the direction of rotation is to the operated side, the eye deviation is slighter than when it is toward the normal side. Furthermore, when the direction of rotation is to the normal side, the eyes sometimes do not snap back from their deviated position (toward the opposite or operated side) until some time after the cessation of rotation, while when the rotation is toward the opposite side (that is the operated side) the post-rotatory jerk back to the normal position takes place immediately upon the cessation of rotation. The post-rotatory jerk, when the rotation has been toward the normal side, sometimes follows only after an appreciable time, its beginning being indicated by a fine tremor of the eye-balls, which are then snapped back to the normal position simultaneously. The most pronounced post-rotatory effect is also observed when the rotation has been toward the operated side.

Removing the stimulating influence of moving retinal images is in agreement with the above description of the rotatory and post-rotatory ocular movements. That is, they are slight during rotation, sometimes not apparent, but very decided when the rotation is stopped and particularly so when the direction of rotation has been toward the operated side.

The reactions of the adults to horizontal tilting are characteristically abnormal. When the container is so tilted that the anterior end of the animal is raised, it responds by lowering its head, during which the deviation and tilting to the operated side are preserved. As the tilting

is continued, the animal sooner or later begins to crawl up, swerving to the operated side, the forelimb on the operated side never being extended so much as the arm on the normal side. When the container is so inclined that the posterior end of the animal is raised, the animal raises its head, extends its arms not so much on the operated side as on the normal side, the head and body thus deviating and tilting to the operated side. As the dish is more and more inclined the animal begins to back and as the grade increases it finally turns sharply to the operated side, sometimes falling over on this side.

If the dish is tilted over to the normal side of the animal, it is not until the tilt of the dish has exceeded 45° that there is any further apparent compensatory movement on the part of the animal because the head and body are already inclined to the opposite or operated side, with the arm on this side markedly flexed, and the other extended. At a greater tilt than this, however, the head is listed further over to the operated side, the arm on this side flexes more while the other is alternately and rhythmically extended (and abducted) and flexed (and adducted) as if seeking for a new and higher support. When the dish is tilted over to the operated side, the animal seems to be incapable of bringing its head to a new horizontal level. The characteristic "pawing" for support does not take place since the influence of the labyrinth on the extensors of this side is absent, and the animal simply rolls over.

Observations on animals deprived of both ear vesicles: *a. Posture.* (Plate I, fig. 3; plate II, figs. 6, 7 and 8.) Although such animals both in the larval and adult stages may be found in apparently normal postures, yet it is only by chance that they come to rest in a normal position after locomotion. These animals can be observed to come to rest just as often on their sides or backs, remaining thus for a considerable period of time, although sometimes when they fall on their back jerky swimming movements are made until an upright position on the bottom is assumed. This is particularly true of the younger larvae (less than 33 to 40 mm.). Older larvae do not continue long in an abnormal position, but turn with a quick flip to the right or to the left. The length of time that an animal would remain in an abnormal posture without struggling was noted in different individuals, and showed that no position seemed more "comfortable" to them than another.

Even after metamorphosis the adults were sometimes found lying as if dead upon their backs when the rounds for feeding were made; and only a jarring of the dish, introducing of food or the application of

strong light would serve to disturb them into active and incoördinated struggling. On the other hand, placing an adult on its back was in many cases followed by an inevitable quick and precise turning over.

If a larva is observed in the normal position on the bottom, the forelimbs are seen to be held in a strikingly abnormal position hanging limply down or back with the palmar surface often upward.

b. Locomotion. Walking is in the nature of a clumsy and incoördinated rolling or lurching. In swimming the animals swerve to right and left, or up and down indiscriminately, as well as spiraling and circling to either side and somersaulting, thus leading up to the abnormal positions of rest described above, sometimes falling on their heads, from which they either drop to an abnormal position or start off again spiraling. The body is in emprosthotonus nearly all the time, particularly during swimming movements. The lunging after and seizure of food is peculiarly clumsy. They may approach food (for example, a bit of writhing earthworm) by stalking movements which are fairly normal (particularly in older larvae) though exhibiting a weakness which causes them to fall over to right or left, but when the final lunge is made the food is seldom caught on account of deflections to the side or upward and a rolling over. When the food is secured, the animal rolls on, landing most often on its back where it chews and swallows. The eyes, however, make it possible for the animals to approach a moving object, such as a bit of food, fairly accurately.

Magnus (11) describes a somewhat similar condition in the monkey from which both labyrinths have been removed. In springing the animal is entirely dependent upon its eyes, the other receptors which enable it to compensate for the loss of the ears, being no longer stimulated as they are when it stands. Before springing the monkey measures the distance and direction of its objective, and after practice the act of springing is carried out with considerable accuracy, but during a sudden and unexpected movement in the air, the monkey is incapable of coördinating.

The feeding reactions of adults may be referred to. When a bit of earthworm, held in a pair of forceps, is offered to one of these animals, in contrast to the responses of normals, the snapping movements are peculiarly incoördinated. The animal sees the food and snaps for it; but its lack of labyrinthine tone is such that the snapping movements lack direction, and the bit of worm is missed more often than not.

The walking of adults is often almost normal until they are fatigued, when they twist and roll from side to side. When placed in water they

swim with no more coördination than they did as larvae. Such larvae hold their heads bent downwards in stalking prey along the substratum, and their swimming movements are characteristically the result of this emprosthotonus, a dolphin roll or forward somersault being the main feature of each spontaneous dash through the water. As adults their emprosthotonus becomes less marked in the resting position, probably due to the increased relative weight of the body upon the limbs, but on post-mortem examination of the preserved animals it was noted that in every case of this type the animal had been killed and fixed in a marked emprosthotonic posture. This was also found, but to a less exaggerated degree, in those deprived of one ear, while in normal animals such a curvature is rare, although not without exception. Whatever may be the weight of such an observation, it is interesting that only in animals where the ears alone were extirpated was there any fixed regular curvature when the animals were dropped into killing fluid.

c. Reactions to rotation and tilting. The animals show no compensatory movements; rotatory and post-rotatory, of head, limbs or eyes, when subjected to rotation on a horizontal turn-table. When tilted out of the horizontal the animals simply slip or fall over after a certain angle of inclination has been passed.

In addition to studying the effects on equilibrium and compensation of the loss of one or both labyrinths, the results of the partial and complete loss of sight were investigated, alone and combined with the loss of one and of both ears.

Observations on animals which have been deprived of one eye. (Plate I, fig. 4.) The following is a brief description of the posture at rest, during and just after locomotion of this type of animal. Walking and swimming seem almost perfect. The only abnormality is a slight list of the head to the side bearing the eye, which is marked when the attention of the animal is drawn to the substratum in the vicinity of food. This list is considerably less than 45° (the typical amount of list toward the earless side) and is hardly noticeable in swimming and crawling, or in the planing-glide following the characteristic swimming lunge which terminates in the typical resting position on the bottom of the dish. The poised and rigid posture of this planing is peculiarly characteristic of the swimming Urodele larva and generally brings out any deficiency of balance in the animal.

Observations on animals which have been deprived of both eyes. (Plate I, fig. 5; plate II, figs. 9 and 10.) There is no observable

loss of balance at rest, nor during crawling and swimming. The list occasioned by the lack of one eye is removed by the loss of the other. The growth of larvae deprived of one or of both eyes is slightly retarded (due to the operation and its healing) but shows at stage 41 a perfect equilibrium.

The early larvae deprived of one eye tend to lie on the operated side, but upon attaining the use of the ears they right themselves and deviate from the normal and balanced posture only when stalking food. At such times the head is tilted toward the eye side to bring the eye into more direct apposition to the substratum.

Of seven animals in which both eyes were successfully extirpated (i.e., no regeneration of either eye), the records show that, although balance became perfected at approximately the usual developmental stage, in feeding these larvae often overshot the mark. The four of these animals that reached metamorphosis exhibited marked opisthotonus when excited. When food was laid in front of them they invariably craned their heads above it, frequently walking over and past it. They also attempted to climb the walls of the dish.

A picture of an adult of this type (see plate II, fig. 10) shows the animal holding its head high with the entire body raised from the ground.

One animal in which both eyes were extirpated, but in which one regenerated so as to form a lens and cornea and to be apparently functional, was recorded as showing a slight list during stalking toward the side of the regenerated eye, and to have straightened out gradually from its former marked opisthotonus as the regenerating eye became complete and supposedly functional.

Observations on animals deprived of one ear and of one eye, on opposite side. The general posture (see plate I, fig. 7; plate III, figs. 11C and 12) is an exaggerated form of that assumed by the animals from which one ear has been removed, that is a deviation toward the earless side. This is true at rest, as well as during and just after locomotion, both in larvae and adults.

Observations on animals deprived of one ear and one eye on the same side. At first glance such an animal appears normal in posture when at rest (plate I, fig. 6; plate III, fig. 11B) so that it may be said that the removal of the eye from the same side corrects for the loss of the ear on that side; when floating or buoyed in water it preserves an even keel. Indeed if such an animal is closely observed, there is seen a definite, though slight, list of the animal, particularly

of the head, toward the side possessing the labyrinth and the eye. Only when excited is there a list to the side lacking the labyrinth and the eye. Thus when the animal swims rapidly the corrective influence of the eye does not exert itself, the animal deviating in the expected manner to the earless side, and swerving to the normal side. When moving along the bottom in the act of foraging for food (crawling) the influence of the eye is again evident (plate I, fig. 6, anterior view), the animal tilting toward the side with the eye and away from the earless side.

Observations on animals deprived of one ear and both eyes. *a. Posture.* (Plate I, fig. 8; plate III, fig. 13.) The asymmetric posture described above for animals lacking one labyrinth is exaggerated here. The deviation of the head, together with its torsion to the earless side is very marked. The forelimb on the earless side is flexed and adducted so that the back of the hand is under the chin (particularly in adults). The opposite arm is extended. The position at rest after metamorphosis was never observed to be normal, as it rarely may be in the animals from which one ear only has been removed. Some of the larvae, however, may assume apparently normal postures when at rest.

b. Locomotion. The loss of the eyes seems to lead to an opisthotonus, particularly in the adults. Therefore, although the animals almost invariably turn over on the earless side when swimming and roll on the longitudinal axis to this side, they do not swerve or circle toward the normal side as do the animals simply deprived of one ear, but toward the earless side. In walking, of course, as in those deprived of one ear only, the adults swerve toward the earless side. The walking of the adults is sometimes very unstable and incoördinated. The animals make quick jerky movements falling over and rolling over to their backs, then floundering and seemingly incapable of righting themselves, and finally lying thus for some time with limbs rather rigidly extended.

c. Reactions to rotation and tilting. Naturally in these animals the possible influence of moving retinal images has been done away with. The responses to rotation are typical of the operation. When the direction of rotation is toward the operated side, the rotatory compensatory movements of head and limbs are very slight and usually absent, the animal often keeping the deviated position. The post-rotatory movements are extremely pronounced. If there has been any rotatory compensation, the post-rotatory movements involve a marked deviation toward the side of the rotation, and a consequent

circling (particularly in larvae) toward this side. There is frequently no rotatory compensation, that is, the deviation, etc., remains toward the operated side, during the rotation toward this side, and then the post-rotatory movement consists in a slightly increased deviation, tilt, etc., toward the same side.

But if the direction of rotation is toward the normal side, the rotatory compensatory movements are exceedingly marked on the part of head and limbs, so that the animal deviates far to the side opposite the direction of rotation (the side lacking the ear). This deviation often culminates in a slow progressive and circling locomotion in the direction opposite to that of the rotation. There is, however, practically no post-rotatory response. Formerly, with the eyes not functioning, the post-rotatory response should be the striking feature. Its failure is undoubtedly to be referred to the loss of function due to the missing ear.

When the dish containing such an animal is tilted so that the anterior end is slowly raised, its head drops in the characteristic asymmetric manner (with the elbows raised, that on the operated side more so), thus deviating and tilting to the operated side, and finally after a certain degree of tilting is passed, the animal crawls up the inclined plane, sharply swerving and jerking its head to the operated side. If the posterior end of the animal is similarly raised gradually, it raises its head, still deviated, etc., to the operated side; and finally, if the tilting is continued, begins to crawl backward and failing this, turns sharply and quickly to the operated side.

When the dish is tilted sideways and downwards on the operated side, there are no compensatory movements on the part of the head and arms, except that just before the animal falls over, the head is sharply bent toward the earless side. When the downward tilting is toward the normal side, there are similarly no compensatory movements except again, that just before falling over the animal jerks its head sharply to the operated side.

It is significant that the single compensatory movement, the "jerk" of the head should in both cases be toward the earless side. It is indicative of the presence of a tonus only in those muscles which would bring about this response, or of a greater tonus than in their antagonists.

These experiments also indicate that the compensatory "pawing" movements are in greater measure dependent upon the eyes and probably have an intimate connection with the deviation of the head in the attempt to keep the horizon level.

Observations on animals deprived of both ears and of one eye: *a. Posture.* (Plate I, fig. 9; plate III, figs. 14 and 15.) The account of the posture, or lack of it, given of the animals deprived of both ears, in general describes that of those in which one eye has been removed in addition. Their chance assumption of almost any position after swimming, and the haphazardness of their resting posture may perhaps be said to be greater, and their righting struggles more prolonged and less successful. But when the animals, by chance, come to rest in their most normal position, ventral side down and all four limbs in contact with the substrate, then there is always a list of approximately 45° toward the side with the eye. This list varies according to the use to which the eye is being put. An animal foraging for food will follow it with its one eye, even though by so doing it tilts over more and more and finally rolls over on its back. These animals are less restless than those lacking merely both ears.

The opisthotonus of these animals is very evident (see plate III, figs. 14 and 15). It must be due to the loss of the eye, since the loss of the ears has generally the opposite effect, that is, the assumption of an emprosthotonic position.

b. Locomotion. In general the locomotion of these animals is quite similar to that of earless individuals, but the general attitude is one of even greater helplessness. Active motions to correct an abnormal position on the bottom, as when bright light is thrown on them, are often ineffective. The swimming of the larvae is extremely vigorous and violent, but with no apparent coördination, the animals rolling, somersaulting at random, until they fall to the bottom exhausted.

c. Feeding. The stalking and engulfing of food seems extremely difficult for these animals to accomplish. In addition to their lacking

Plate III. Photographs of various operated types, taken from life in their characteristic resting postures. Orientation of the animals to the camera is uniform throughout all the figures and is the same as in plate II.

Fig. 11. Comparative study of the resting postures of the three different single-ear extirpations. *A* lacks the left ear; *B* lacks the left ear and left eye; while *C* lacks the right ear and left eye.

Fig. 12. Adult, lacking right ear and left eye.

Fig. 13. Adult of 10 months, lacking the right ear and both eyes.

Figs. 14 and 15. Larvae having both ears and the right eye missing.

Figs. 16, 17 and 18. Larvae deprived of both their ears and both their eyes.

Figs. H and J. Two postures taken commonly by a young adult Rana sylvatica from which the right ear had been extirpated in the early tail-bud stage of embryonic life.

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both labyrinths, they have but one eye and when they attempt to follow an object with this, as above described, so as to keep it in view, the result is a total loss of equilibrium. This asymmetry of movement makes the procuring of food next to impossible, a fact evidenced not only by watching them attempt it, but also by the fact that they grow very slowly and metamorphose much later than all others. These animals must be literally fed, the bits of food being offered to them repeatedly.

In cases where both ears are absent in addition to one or both eyes, the commonly called forth excitement-rigidity of the body takes the form of opisthotonus. This occurs throughout life and was noted frequently as a distinctive posture of these groups. Opisthotony was not, however, observed to any regular degree in the fixation of this group.

It may be concluded from this general evidence that the opisthotonus resulting from the loss of the eyes affects very little the tonus of the trunk muscles and has its chief effect upon those muscles poising the head on the neck. Whereas the removal of the ears produces a marked emprosthotony of the neck and trunk, which is more or less evident according as to whether both ears, or only one, have been removed.

Observations on animals lacking both ears and eyes: *a. Posture.* (Plate I, fig. 10; plate III, figs. 16, 17, 18.) Balance is entirely lacking, and a normal upright position is come upon merely by chance. This is in part due to the lack of visual stimuli, since the animals with eyes (without ears) are often in normal positions. It is also indicated by the observations that in darkness animals deprived of both ears, but having both eyes, were about as often to be found in haphazard positions as these animals are when in diffused daylight. These chance positions are kept for indefinite periods of time, and these animals show additional evidence of being different in that they will accept food, chew and swallow it without attempting to right themselves.

b. Locomotion. Due to the usual, though not constant, opisthotonus, these animals nearly always swim in circles on one side or the other and swerve toward the dorsal side. When search after food is made, it is done with the aid of the olfactory and tactile sense. The animals may, and do, approach their food fairly accurately, but they do not lunge after it, and snap for it only after they have come in actual contact with it. The movements of walking are similar to those described for the earless individuals. To horizontal rotation and to tilting there is no compensation.

OBSERVATIONS ON TADPOLES AND YOUNG ADULTS OF RANA SYLVATICA, FROM WHICH ONE EAR HAS BEEN REMOVED. One ear was removed from a number of frog tadpoles (3 mm. long). Six survived to the time of the appearance of hind limbs, and three of these metamorphosed, the other three being lost during absence from the laboratory.

The behavior of these animals agrees in the main with the description given by Streeter (3), in that they lean to the operated side, roll on the long axis in swimming (toward the operated side) and spiral and circle. But the tilt to the operated side is always pronounced as evidenced by the position of the dorso-caudal fin. While there is some slight recovery from the disturbances in equilibrium and of locomotion, these animals would never, either at rest or in swimming, be mistaken for normal tadpoles.

Streeter found that with the beginning of function of the posterior limbs the abnormality in swimming was less pronounced. We have not been able to substantiate this finding and our experience has been that these animals are always very abnormal while swimming. Upon metamorphosis this abnormality in posture is in no wise lost. At rest the animals are tilted far over to the operated side, the fore limb on that side is flexed under the head while the opposite limb is extended. The back is curved, although the position of the posterior limbs differs but little from the normal. On the whole, the general posture of the animals is similar to that usually described for frogs lacking one ear (cf. de Kleijn (12)). (See plate III, figs. H and J.)

If the animals are observed as they cling to a vertical wall to which they have jumped, their abnormal posture is very striking. Instead of facing directly upwards they make an angle with the vertical, bracing as it were to the sound side of the vertical, the operated side being closer to the wall with the limbs on that side flexed, while the opposite limbs are extended, thus supporting the body.

The swimming and jumping movements are typical of adult frogs from which one ear has been removed or injured, the abnormal position of the limbs and the head being never lost so long as the animals lived.

The results of rotation experiments are similar to those given above for Amblystoma deprived of one ear as well as for operated adult frogs.

SUMMARY

1. Normal Amblystoma when rotated horizontally, give typical rotatory and post-rotatory reactions of the head, trunk and limbs. On slow rotation characteristic eye movements are also observed.

Inclination of the horizontal plane of the animal results in the assumption of compensatory positions of the entire body.

2. The posture of an animal from which one embryonic ear has been removed is throughout life abnormal; the deviation and torsion of the head always persisting, as well as the asymmetrical limb positions. In locomotion the animals turn over to the earless side with a greater or less swerving toward the normal side. When locomotion is vigorous, the animal either rotates on its long axis toward the earless side, or swims in narrow circles, with the earless side under, toward the normal side. Rotatory and post-rotatory compensatory reactions are accentuated or diminished in accord with the direction (toward the normal or the earless side) of the rotation. While each labyrinth has a binocular influence, there is some evidence of a reduction in tone of the ocular muscles when one ear has been removed. Inclination of the horizontal plane of the animal accentuates the list and deviation of the animal to the opposite side alone.

3. Animals deprived of both ears may come to rest in any chance position although they show a tendency until fatigued to struggle upright. Locomotion is clumsy and incoördinate, indicative of a lowered muscular tone. Crawling, as after food, may be fairly accurate owing to the ocular sensations. There are no compensatory reactions to rotation or tilting. The removal of one or both ears usually causes a slight emprosthotony. This explains the general tendency in swimming to swerve toward the normal side.

4. The only abnormality involved by the lack of one eye is a slight rotation of the head on its long axis toward the side bearing the eye. This is accentuated when the animal is attentive to the substrate.

5. The outstanding characteristic result of removal of both eyes is the assumption of a slight opisthotonic position when the animal is poised or in vigorous locomotion.

6. Removal of a single ear and its opposite eye exaggerates the equilibratory defects occurring when merely one ear is missing.

7. Conversely the removal of the eye from the earless side has a corrective influence and the balance is normal except during vigorous swimming.

8. Eyeless animals from which one ear has been also taken show the loss of the ear to a more marked degree than when the ear alone has been removed due to opisthotonus and asymmetric posture. Rotation and tilting of such animals initiate very limited and characteristic compensatory movements toward the earless side of the body alone.

9. Animals devoid of ears and with only one eye are the most helpless of all, their tendency to opisthotonus being continuously noticeable.

10. Specimens entirely devoid of ears and eyes are incapable of sustaining their balance or acquiring an upright position except by chance. They make no compensatory movements to rotation or tilting.

11. Tadpoles of Rana sylvatica from which one ear vesicle has been removed show throughout larval life and for several weeks after metamorphosis the abnormalities of posture, locomotion and reaction to rotation and tilting characteristic of the operation.

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Paramecium polycaryum, sp. nov.

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The several species of the genus Paramecium naturally fall into two groups: one with the general cell form represented by *P. aurelia, P. caudatum,* and *P. multimicronucleata* (aurelia group), and the other by *P. bursaria, P. putrinum* and *P. calkinsi* (bursaria group). Within each of these groups the species are distinguished chiefly by micronuclear structure and number. One type of micronuclear structure (caudatum type) occurs in *P. caudatum* and *P. bursaria*, and *P. putrinum;* the other (aurelia type) in *P. aurelia, P. multimicronucleata* and *P. calkinsi.* Species with the "caudatum type" possess a single micronucleus while those with the "aurelia type" possess two (*P. aurelia, P. calkinsi*) or several (*P. multimicronucleata*).[1]

The present paper records the discovery, on November 29, 1922, in some material collected in Louisiana, of a Paramecium characterized by the general body form of the "bursaria group" and the micronuclear structure of the "aurelia type," but possessing several (three to eight) micronuclei. In brief, the organism is essentially identical in form and structure with P. *calkinsi,* but has more than two micronuclei. Thus from the

[1] L. L. Woodruff, *Biol. Bull.*, 1921, xli; Proc. Soc. Exp. Biol. and Med., 1921, xviii.

standpoint of micronuclear structure and number this animal holds the same position in the "bursaria group" as *P. multimicronucleata* in the "aurelia group."

Pedigree cultures from the original animal found have now been under observation and experimentation for nearly four months, and through upward of one hundred and fifty generations. During this time the animals have bred true, exhibiting the characteristic micronuclear number after emerging from the nuclear reorganization involved in endomixis.

In view of all the above data the animals of this culture are designated a new species, *Paramecium polycaryum*.[2]

2 Details of the structure and life history of *P. polycaryum* will appear in the *Biological Bulletin*.

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ON FAMILIES OF SPIDERS

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Taxonomy is the mirror of Evolution. In it the relationship of forms is reflected, bringing us face to face with results of past processes, just as Physiology and Genetics reveal to us inner activities of living organisms. It is the supreme effort of the human mind to bring into a natural system knowledge derived from the study of Morphology in its widest sense. Gross and microscopic anatomy, embryology, behavior and instinct, paleontology, geographical and geological distribution—nay, even physiology and chemistry—contribute to the building of the system wherein the breadth and depth of our knowledge find their expression. Only limitation of knowledge makes the system imperfect, only limitation of mind makes some present-day zoologists relegate taxonomy to the dust-heap of accumulated facts not worthy of further examination. As in a poor mirror the image may be distorted, so in a system built up by a taxonomer the relationships may be wrongly represented. The smaller the knowledge, the greater the distortion. Yet the study of taxonomy has this advantage, that even a wrong step is a step forward, for it reveals to us something that until then had escaped our observation, something that may possess special significance. Thus parallelism and convergence in development for a while were mistaken for true relationship, but are now of help in the understanding of directions in evolution.

Long before evolution became the object of discussion, taxonomy was trying to establish relationships of forms. Viewed from a distance of several decades of study, not only the system of Linnæus, but those of Cuvier, of Latreille, and even of Huxley and Haeckel, appear to us crude and unnatural. Yet, if regarded in the light of contemporary knowledge, they embodied the latest discoveries and represented a great advance over what was known previous to them. It is not my object here either to discuss these systems, or to give a system of my own which would embody our present knowledge and cover the entire field of animals. Such a task is now beyond human power, for first-hand knowledge of taxonomy requires so much time to acquire that one can possess it only in the case of a smaller group. In another paper I have touched upon the relationships of the various orders of Arachnida

among themselves and with other classes of Arthropoda. Here I intend to discuss the order of spiders, which I have been studying from many points of view for many years past.

Needless to say, all old systems are quite out of date Even such an illustrious student of spiders as Walckenaer did not have a clear conception of families and did not know the value of characters. He was the first, however, to apply to his system characters derived from the study of habits, locomotion, nest-making and snare-construction. This system, somewhat modified, was in vogue for many years afterwards. It found its application even in the system of Thorell and has resulted in the approximation of such different forms as Uloboridae and Epeiridae, Dictynidae and Theridiidae, Amaurobiidae and Agelenidae. An earlier attempt by Latreille to divide spiders into Tetrapneumones and Dipneumones, based on the number of book-lungs, later followed by Bertkau and many other araneologists, led Dahl to the separation of the Hypochilidae from the sub-order to which it naturally belongs, on the ground that spiders of that family have four book-lungs.

Many attempts have been made during the past fifty years to create a comprehensive system, and invariably the limits of established groups were obliterated by the widening of our acquaintance with exotic and especially with tropical faunas. Our present system is almost entirely due to the genius and unsurpassable knowledge of Eugène Simon. It is embodied in the second edition of his Natural History of Spiders and represents the results of an analysis of a vast number of species and of patient labor extending over forty years. With slight modifications, it is generally accepted and all attempts at change are directed chiefly against minor points in the system. Yet it is far from perfect. Simon makes the persistent attempt to widen the limits of genera and of families, because he finds so many intermediary forms that all lines between groups become effaced. We see this in the recognition of a single family, Avicylariidae, instead of three or four, as is done by Pocock and others; in the inclusion of the Deinopinae in the Uloboridae; in the fusion of the Linyphiidae with the Epeiridae and Tetragnathidae into one immense family, Argiopidae; in a similar fusion of groups in Thomisidae, and perhaps quite especially in the establishment of a single family, Clubionidae, for such diverse forms as Selenopidae, Ctenidae, Heteropodidae, Clubionidae. We see the attempt to widen the limits of genera in the merging of a number of genera in the single genus *Araneus* which I myself saw was necessary to treat as a cohors in my Catalogue; in a similar merging of several genera into one genus *Dendryphantes*, and again in the case of *Lycosa*. Frederick P. Cam-

bridge, who did so much during his brief span of life for the elucidation of natural relationships in spiders, made a praiseworthy attempt at the delineation of more limited genera in several instances, but without much success. Such genera or cohortes as *Araneus, Dendryphantes,* etc., require extensive study and a natural division into genera may be accomplished only when material from all over the world is taken into account. All attempts at classification based on limited faunas are invariably unsuccessful, applicable only to the particular fauna and therefore misleading when it comes to comparisons. Several studies of limited groups were made within recent years by various araneologists, Simon, Cambridge, Dahl, Peckham, Comte de Dalmas, Fage, Chamberlin, Mello-Leitâo, but the field is still open to extensive investigations which would be most welcome.

The great difficulty in delimiting families lies in the value which one accords to each character. Simon, for example, entirely omits to mention the taxonomic value of trichobothria, perhaps because he does not attribute any particular value to them, or perhaps because we still lack sufficient comparative knowledge of these sense organs. On the other hand, Dahl goes so far as to base a great deal of his classification on the distribution of trichobothria which he calls "auditory hairs." Following this clue, he separates Simon's Thomisidae into several families, and in fact establishes the new sub-orders Chalinurae, Oligotrichiae, Stichotrichiae, and Polytrichiae, each embracing several of Simon's families, but not in any way coinciding with Simon's sub-orders. I believe that the value of this character is greatly overestimated by Dahl, but I also believe that we are indebted to him for having pointed out to us a character which may be used to advantage in special cases.

And here, then, naturally arises the question, What is a family-character? Can any structure that distinguishes one group of animals from another group be reasonably considered a family-character? Evidently not, for otherwise we should immediately commit the error of uniting convergent forms in one family, though their entire pasts were quite different. The reader may dissent on the ground that the object accomplished, not the process by which that object was accomplished, is of primary importance; that water is water, whether we get it by condensation of vapor or melting of ice, by distillation from a chemical solution or by the combination of hydrogen and oxygen atoms in the presence of an electric spark. But an organism is not a simple chemical and it carries within it the marks of its origin. Even where such marks are almost entirely obliterated, as in some parasitic Crustacea, embryology reveals traces of them. But should even the last marks be lost, the

species would, nevertheless, be distinct, though we could not grasp the distinction with the aid of our existing methods, and would cease to be a distinct species only when and if it should become absolutely identical with some species of another group. This, of course, to our knowledge has never happened.

Have, then, the trichobothria such value that the various families composing Simon's division Cribellatae, may be split into four groups and, together with ecribellated spiders, placed under the above-mentioned suborders, as Dahl has done? If we remember that the trichobothria are sense-organs and, moreover, sense-organs of the lowest type, for they are nothing but modified tactile hairs, we must expect to find an inclination in that character to great variations. And so it is and it would be entirely out of harmony with all other sense-organs if trichobothria were permanent or even, generally speaking, dependable characters. Take, for instance, the eyes; they belong to two different somites, two neuromeres of the brain supplying them and them only with nerves. And yet eyes vary in number in closely related species, the variation being sometimes directly traceable to cave-dwelling habits. The auditory function of the trichobothria is far from having been demonstrated in a way precluding all controversy; but without going into the discussion, treating them broadly as a type of tactile organs, why should they be exempt from variation when they receive their nerve supply from five different neuromeres and when they share that nerve supply with a number of other organs in the same limb?

If we pause for a moment to think, we will come to the conclusion that in the process of evolution some characters must have been subject to greater variation than others, since otherwise all resemblance between the forms would have been lost at the start. These more stable characters will be present, therefore, in a greater number of forms, and in the sequence of time will have the value of class-characters, order-characters, family-characters, and so on. Examine the mutation-characters observed or produced in breeding experiments during the last decade. Not one of them has a greater value than that of a sub-species or at best of a species. Even generic characters remain unaltered, not to mention family-characters or order-characters. This shows that most of the changes occur, so to say, on the surface, leaving the substratum intact. It follows that if we could find the exact extent of variability for every character of a number of species, we could almost infallibly decide the taxonomic value of each character. Unfortunately, instead of welcoming variability and making it a subject of particularly painstaking study, the systematist usually either disregards it as something of no particular

value or, what is equally objectionable, tries to make new species out of the variations. There is actually not a single investigation on the variability of characters in spiders which would treat the subject from a comparative point of view and on the basis of study extended over a large field of forms and a sufficiently great number of specimens. What there is, is casual and therefore inadequate. A little work has been done on the variability of the number of teeth in claws, and also of teeth on the margins of chelicerae. It has been shown that there is a greater constancy in the latter than in the former. It has been also shown that the number of teeth on claws varies with the age of the spider, somewhat changing after every molt. Spiderlings emerging from the egg are in some cases completely naked, while later their integuments become clothed with hair. Some spiderlings possess three claws, the single claw disappearing in later molts—an evidence that the original number of claws must have been three. As a consequence, we find a rudimentary third claw in Paratropididae and some Theraphosidae, and we find three claws on the posterior legs and two claws with a rudimentary third claw on the anterior legs in some Tengellidae.

Generally speaking, family-characters should be derived only from structures as they appear in very young spiders. But here again we strike a great difficulty. Young spiders cannot be identified except in very few common species, and the changes due to age cannot be verified. In the case of diverging development, this is not so bad. I have shown, for example, that the sternum of the scorpion *Centrurus is* pentagonal in the young and only later becomes triangular. This merely tends to prove that the pentagonal shape is the original one, and therefore the consequent change to the triangular shape has additional value. But in the case of converging development we should be quite misled. And in both cases, in identifying the young and the adult of the same species, we should be liable to place them in two different families, being ignorant of the changes due to age. The present system of spiders is based entirely and exclusively on characters of mature individuals and already for this reason alone is imperfect. Nevertheless, a classification has to be given and has to express relationship. Let us therefore consider the characters on which such a classification may be based.

Simon bases his first division on the articulation of the chelicerae. He thus establishes two sub-orders, Araneae Theraphosae and Araneae Verae, and refers the family Liphistiidae to the former. Pocock separates the Liphistiidae from all other spiders. He puts them in the sub-order Mesothelae, as opposed to the Opisthothelae, which he divides into two sub-orders, the Mygalomorphae and the Arachnomorphae. Finally,

Dahl divides the order into the following sub-orders: Verticulatae, synonymous with Pocock's Mesothelae; Tetrapneumones, including besides Pocock's Mygalomorphae also the family Hypochilidae (which both Pocock and Simon place among the Cribellated spiders of the sub-order Arachnomorphae or Araneae Verae); Apneumones for the family Caponiidae; Chalinurae for the family Hersiliidae; Oligotrichiae for the families Dysderidae, Sicariidae, Urocteidae, Uloboridae, Tetragnathidae, Palpimanidae, Filistatidae, Dinopidae, Eresidae, Dictynidae, Mimetidae, Archaeidae, Theridiidae, Pholcidae, Araneidae, Micryphantidae, Linyphiidae; Saltigradae for the family Salticidae; Laterigradae for the families Strophiidae, Platythomisidae, Misumenidae, Diaeidae, Amyciaeidae, Mystariidae, Philodromidae, Urarachnidae, Bomidae, Xysticidae, Aphantochilidae and Zodariidae; Stichotrichiae for the families Stephanopodidae, Hahniidae, Amaurobiidae, Agelenidae, Psechridae; Citigradae for the families Senoculidae, Ctenidae, Zoropsidae, Lycosidae and Zoridae; Polytrichiae for the families Argyronetidae, Oxyopidae, Tengellidae; and Tubitelae for the families Ammoxenidae, Prodidomidae, Platoridae, Gnaphosidae, Zorocratidae and Clubionidae.

Pocock's idea in separating the Liphistiidae in a special sub-order Mesothelae is undoubtedly sound, although the name chosen by him is misleading and therefore objectionable. The ancestors of spiders had originally a segmented abdomen composed of twelve somites. In the course of time the first abdominal somite became very small and assumed the shape of the pedicel or petiolus as we see it now. The second abdominal somite bore typically the genital opening and also a pair of lungs. The third somite bore the second pair of lungs. Of the pleiopodes or abdominal appendages of the following somites only those of the fourth and fifth somites remained, while the others have early disappeared and are visible only in embryonic stages. These appendages were biramous, as in lower Arthropoda. They became modified as spinnerets, each ramus became more or less independent, and that is the reason why we find in Liphistius two pairs of spinnerets on the fourth and two pairs on the fifth somite. The anus was on the twelfth somite in the ancestors of spiders, but moved one or two somites forward with the disappearance of the twelfth and eleventh somites. Thus in *Liphistius* the anus is separated from the spinnerets by several somites. With the complete loss of segmentation in other spiders, all somites posterior to the fifth were also lost and are at present represented by the anal tubercle, which therefore comes to lie immediately behind the posterior spinnerets. The name Mesothelae is wrong because the spinnerets have not moved from the fifth somite either in *Liphistius* or in the other

spiders. To correct this error and to make the names more uniform, I shall call this first sub-order Liphistiomorphae.[1] Its chief morphological character, then, lies not in the fact that the abdomen is segmented or that the spinnerets are considerably in front of the anal tubercle, but in the fact that posterior to the spinnerets the abdomen is still composed of several segments. This sub-order may be divided into three families, two of which are Carboniferous (Arthrolycosidae and Arthromygalidae) and one recent, Liphistiidae. In my monograph of Palaeozoic Arachnida I referred all carboniferous segmented spiders to a single family, Arthrolycosidae. It seems to me now that the arrangement of the eyes in the American species *A. antiqua* and *A. danielsi* is so different from that in the European species that the establishment of the new family Arthromygalidae is warranted. The Arthrolycosidae cannot be at present well defined. We do not know the number of their claws, nor the structure of their spinnerets, nor of their sternum. Nevertheless, considering the remote geological period at which they lived, we may be safer in keeping the family than in merging it with the Liphistiidae. The genera *Protolycosa* and *Eocteniza,* from the Carboniferous, possibly also belong to the Arthrolycosidae, although the arrangement of their eyes is not known.

The Arthromygalidae are easily distinguished both from the Liphistiidae and the Arthrolycosidae by the arrangement of their eyes in two rows and by the presence of two smooth claws on their tarsi. The new genus *Arthromygale* includes three species from the Carboniferous of Europe, namely, *A. carbonaria, A. fortis* and *A. beecheri,* all described under the generic name of Arthrolycosa, while the systematic position of *Arthrolycosa lorenzi* and *A. palaranea* remains uncertain. The genus *Rakovnicia,* with the single species *R. antiqua,* probably also belongs to this family, while the position of the genera *Geralycosa* and *Perneria,* all from the Carboniferous of Europe, is less certain.

The presence of eight spinnerets in Liphistiomorphae may be regarded only as a subsidiary character, since a reduction in the numbr of spinnerets may take place independently of other characters and would be quite compatible with the persistence of segmentation posterior to the fifth somite.

One might regard all spiders with an unsegmented abdomen as belonging to a sub-order equivalent in value and opposed to the Liphistiomorphae. Without calling such a group a sub-order, Pocock has applied to

[1] I do not believe that the law of priority should interfere with a new name for a sub-order in such a case, and at any rate the name Arthrarachnae, proposed by Haase, has priority over Mesothelae.

it the name Opisthothelae. This, however, seems to me objectionable for two reasons: First, it would necessitate the creation of divisions under the sub-order, and, second, it would be reasonable only if the composition of the abdomen in all non-segmented spiders were the same. This, however, is not only far from being certain, but may altogether not be true. It is, for example, known that the heart in Arachnomorph spiders has three pairs of ostia, while in Mygalomorph spiders it has four pairs. The number of ligaments suspending the heart seems also to be different. All this may point to the possibility that in the abdomen of Mygalomorph spiders the sixth somite is still present.

Be this as it may, the separation of the Mygalomorphae from the Arachnomorphae would be clear and simple were it not for the family Hypochilidae, which has the appearance and the cheliceral articulation of Arachnomorph spiders and at the same time possesses four lungs, as in Mygalomorphae.

Are the Hypochilidae Mygalomorph or Arachnomorph? We have seen that Dahl unites them with the Mygalomorph spiders in a sub-order, Tetrapneumones (therein he is following some of his predecessors), while Pocock and Simon consider them to be true spiders. A study of the circulatory system and of the coxal glands would probably decide this question beyond controversy. But, as no such study has ever been made, it becomes largely a matter of opinion as to which of the known characters to consider the more important ones. We know that in the tremendous change that involved the posterior somites in Mygalomorph spiders the number of lungs remained the same as in the Liphistiomorphae. We know that in true spiders two families have no lungs at all, but in their place have a pair of tracheal tubes. All this makes one think that the type of breathing organs is rather a functional adaptation and, taken by itself, does not possess the value of a fundamental character. Moreover, the Hypochilidae possess a cribellum and a calamistrum, structures not found in Mygalomorph spiders. Except for the possession of four lungs, the Hypochilidae would appear entirely isolated among the Mygalomorphae, while they have a number of characters in common with true spiders. I therefore do not hesitate to leave the Hypochilidae among the Arachnomorphae until better evidence is furnished for their removal from this sub-order.

If it will ever be possible to confirm my supposition that in the Mygalomorphae the sixth abdominal somite persists throughout life, then this would be indeed the fundamental character for the separation of the Mygalomorphae from the Arachnomorphae. At present we have to content ourselves with more evident characters. Among these the chief

characters of the Mygalomorphae are: the presence of eight ostia (four pairs) in the heart; the presence of two pairs of coxal openings, the first pair behind the first coxae, the second pair behind the third; and the articulation of the chelicerae permitting only an up-and-down motion of the fang. In consequence of this type of articulation the Mygalomorphae strike and then mash their prey instead of biting it.

Within the order Mygalomorphae there is again a divergence of opinion as to the number and scope of families. Simon recognizes just two, the Avicularliidae, with 9 sub-families embracing almost all known Mygalomorph spiders, and the Atypidae, with a single sub-family and only two genera. (Originally Simon placed the sub-families Brachybothriinae and Hexurinae among the Atypidae, but in the supplement to his work he removed them to the family Avicularliidae.) Dahl apparently follows in this respect Simon's example, while Pocock and Cambridge recognize four families: Theraphosidae, Barychelidae, Ctenizidae and Atypidae. There is therefore no dissension as to the family Atypidae. How about the other families?

Simon's conception of the family Avicularliidae is, to my mind, too broad, and the family is not only out of all proportion if compared with the Atypidae, but presents groups within its own limits no less distinct from each other than they are from the Atypidae. The maxillary lobes of true spiders contain special glands the secretion of which seems to form part of that fluid which is used in predigesting the food. The absence of maxillary lobes in Mygalomorph spiders may have direct relation to their method of mashing and eating, not predigesting and then sucking their prey. Nothing is known about the maxillary lobes of the Atypidae. They may have maxillary glands or they may be merely apophyses more developed than those of some other Mygalomorph spiders. On the other hand, we observe a remarkable relationship between the habits and the structure of the legs and spinnerets in spiders. The habits should not be looked upon as being of little importance. Taking the females alone into consideration, since the males show instincts modified under the influence of their method of copulation peculiar to all spiders, we find vagrants who hunt their prey in the open and seek shelter in crevices, under rocks or fallen trees; burrowing species making deep burrows in the ground unprotected by any structure of silk; tube-weavers in the ground and above the ground, with or without a trap-door; and finally funnel and basketweb-weavers of various kinds. Especially the latter differ in every respect from all others.

I have purposedly arranged the habits of the spiders in the above order because it is the usually accepted order in which the more complicated

habits are supposed to have been developed from the more primitive ones. It seems to me, however, that this sequence is not correct, and that changes took place in two different directions, resulting in specialization and complication on the one hand and in simplification on the other. The ancestors of Mygalomorph spiders possessed probably three claws, were sparsely covered with hair, had no scopulae on their legs and lived as simple burrowers. Some of them became expert diggers and weavers of trap-door nests, developed a rastellum on their chelicerae and lost a great deal of their hair. I think that Simon's sub-families Ctenizinae, Actinopodinae and Brachybothriinae are all representative of this type and may be justly united in a single family, Ctenizidae. The Migidae represent a further development, while the Atypidae may have had to adapt themselves to the conditions found in more moderate climates. We know nothing of the life of the Paratropididae, but their characters warrant their elevation to the rank of a family. Some of the descendants of the original ancestral stock took to a more or less vagabond life. They developed hair on their tarsi and metatarsi, lost the third claw and with the aid of the more or less thick hairy clothing of their body became more fit to meet the exigencies and greater dangers of exposed life. In the Barychelidae we actually have transitional forms between skilled burrowers and tube-weavers on the one hand and simple burrowers and vagrants on the other, as are some Theraphosidae. Finally we have the funnel and basket weavers in the Dipluridae. This family may be divided into three sub-families, Diplurinae, Hexurinae and Dolichosterninae, the last containing considerably aberrant forms from Queensland described by the late W. J. Rainbow, together with R. H. Pulleine. Mr. Chamberlin places under the family Dipluridae also a new sub-family, Pycnothelinae, based on the new genus, *Pycnothele,* from Brazil, and according to him enclosing also Thorell's genus *Lycinus.* The characters of *Pycnothele* preclude, however, their inclusion among the Dipluridae. They have only two claws, and these with two series of teeth and their spinnerets remind more those of Barychelidae than of Dipluridae. For this reason I propose to separate this genus and to create for it a new family, Pycnothelidae. Altogether we may therefore recognize eight families of the sub-order Mygalomorphae.*

The sub-order Arachnomorphae presents at the very start great difficulties. We have already partly discussed the case of the tetrapneumone

* In a monograph on the Theraphosidae of Brazil, which has been published after the completion of my manuscript, Mello-Leitão gives the Paratropididae the rank of a family and places into this family the Pycnothelinae as a subfamily. I see no reason why I should modify my opinion in this regard and prefer to retain Pycnothelidae as a family.

family Hypochilidae. We have seen that except for that character the Hypochilidae are to all purposes Arachnomorph spiders. But how about the cribellum and calamistrum themselves? For years they were not considered to be of more than generic importance. *Amaurobius* was classified with the Agelenidae, *Dictyna* with the Theridiidae, *Uloborus* with the Epeiridae and so on. It has been pointed out by various authors that there is a great deal of similarity in appearance, structure and habits among many spiders otherwise readily separable by the presence or absence of the cribellum and calamistrum. *Psechrus* and *Tegenaria, Themacrys* and *Tegenaria, Acanthoctenus* and *Acantheis, Amaurobius* and *Coelotes, Filistata* and *Plectreuris, Oecobius* and *Uroctea* represent such species. We have similarities between the Zoropsidae and Zoridae, Drassidae and Agelenidae; between the Dinopidae and Pisauridae and Oxyopidae; between the Eresidae and Attidae. These similarities are now usually treated as examples of parallelism or converging development. Yet even such a careful student of characters as Frederick P. Cambridge, who separated Cribellates from Ecribellates in his great volume on Central American spiders, was frankly puzzled and on page 352 writes:

> Two theories may be advanced: (1) The cribellum, specialized as we now know it, is a very ancient organ, antedating, not only in its origin, but in its actual functional specialization, the division of the Araneae into the various broad families at present recognized. If we hold this view, we may regard all the spiders in which the *cribellum* is now specialized as the offshoots of some one or more ancentral forms, divergent in this particular respect from the main body in which no specialized cribellum was developed. . . . If this theory be accepted, with the assumptions necessary to its consistency, then we shall, as Simon has done, divide the Araneae into "Cribellatae" and "Ecribellatae." But if not, there is an alternative theory (2), that the cribellum is a comparatively recent specialization of the anterior surviving pair of abdominal appendages possessed originally by all early forms, at any rate long postdating the conditions under which the divergence into the present groups was initiated. . . . We are thus compelled to choose between the probability of a convergence in general characters amongst members of originally widely divergent families, or the probability of a sporadic specialization of two appendages (possessed by all the members of the original phylum) cropping up here and there and themselves structurally converging towards a very similar type. Personally, I am of opinion that the probability lies with the second theory and that evidence also tends strongly in this direction; and although it might be much more convenient, as in the first case, to place the "Cribellates" together as distinct from the "Ecribellates," I cannot believe that this course furnishes as natural a classificataion as that represented in the second case. It seems more probable that the calamistrum and cribellum have been independently developed and specialized in different families than that

members of groups ancestrally widely divergent should have converged and assumed general characters so similar as to be scarcely distinguishable from each other.

On page 356 Cambridge writes of the family Psechridae that "in all probability they constitute a cribellate form" of the Agelenidae. On the other hand, on page 360 he writes of the family Uloboridae:

The spiders comprehended in this family probably have no near relations amongst existing forms; so that it is difficult to place them in any system of classification. They represent possibly the survivors of larger groups, the majority of whose members have long since died out.

And yet Koch, Thorell and McCook did not hesitate to consider the genera *Uloborus* and *Hyptiotes* as belonging to the family Epeiridae.

Dahl does not attribute great value to the cribellum and calamistrum. We have seen that he relegates the Hypochilidae together with the Avicularüidae and Atypidae to the sub-order Tetrapneumones. This alone would show that he considers the possession of a cribellum and calamistrum as a subordinate character and the structures themselves as of later and independent origin. We find further evidence of this attitude in the separation of the various cribellate families and their inclusion under different sub-orders. The Zoropsidae which Dahl once treated as a single family he now has split into three families. Of these the Zoropsidae, containing, according to Dahl, a single genus *Zoropsis,* he places in the sub-order Citigradae, the Tengellidae in the sub-order Polytrichiae and the Zorocratidae in the sub-order Tubitelae. Moreover, Dahl puts the Oecobiidae as a sub-family under the family Urocteidae and the Acanthoctenidae as a sub-family under the family Ctenidae. If a taxonomic system is not an arbitrary separation of forms on the basis of characters which have no value whatsoever, but represents an attempt at expression of true relationship, and if we analyze this classification of Dahl's, we must come to the conclusion that the separation into his sub-orders anteceded the formation of the cribellum and calamistrum and that therefore Dahl is in favor of the second theory mentioned by Cambridge.

Have the ancestors of Arachnomorph spiders possessed two pairs of biramous pleiopods, as the recent Liphistiids still possess them? Have some of them then developed a cribellum from the endopodites of the anterior pair of pleiopods, while others either entirely lost these endopodites or changed them into a colulus? Have these two types then developed independently with convergence in other characters due to functional adaptations? Or is the cribellum the original structure and

have the Ecribellatae gradually developed from various families of the Cribellatae? Or is, as Cambridge states, the cribellum a new structure independently developed in different families?

The last two questions must be carefully kept apart, for the answer to them would lead us to very different conceptions in classification. Suffice it to mention that in the one case the Oecobiidae would have developed from the Urocteidae and the Acanthoctenidae from the Ctenidae, while in the other case the Urocteidae would have developed from the Oecobiidae and the Ctenidae from the Acanthoctenidae.

Paleontology unfortunately gives us no clue whatsoever to the solution of all these questions. Arachnomorph spiders seem to have existed already in the Carboniferous, but the number of species and, in fact, of specimens which are known to us is so small and the specimens themselves so imperfectly preserved that we do not know whether those spiders had a cribellum and calamistrum or were ecribellate, whether the number of their spinnerets was already six or still eight, as in the Liphistiomorphae. We know spiders again only from the Tertiary. The American specimens are too imperfectly preserved to give us a fair idea of the structure of the spinnerets and hind-legs in every case. But the spiders from the Amber of the Baltic and some species from Switzerland and France are so well preserved that one may discern all the most important structures. We can state, therefore, positively that in the Tertiary both Cribellatae and Ecribellatae were already represented and that quite a number of families were already well defined.

We have, therefore, to seek an answer elsewhere, and first of all from the evidence furnished by embryology. Although a great deal remains still unknown and should be investigated, and other things are not convincing and should be reinvestigated, yet on the whole we have a fairly good picture of developmental changes. It is fairly safe to assume that structures which are common to several families and which appear early in the development represent ancestral characters antedating the separation of the ancestral stock into the present families. At the same time it is quite possible that some character having more general value, and therefore greater stability than other characters, undergoes modification at a later period. But it is more than doubtful whether so-called atavistic reversals to ancestral characters can ever take place if such characters have once been permanently lost. In examining developmental changes of spiders we find that the cribellum develops from the endopodites of the abdominal pleiopods of the fourth abdominal somite. They are really the pair of spinnerets still in existence in *Liphistius*, modified, but functioning in all cribellated spiders. In forms with a divided cribellum

the change has not yet progressed so far as in forms with an entire cribellum. The latter is a later stage in evolution. In such genera as *Dictyna* and *Acanthocephalus* we still find species with a divided cribellum and such with an entire cribellum. The colulus is only a further step in the disappearance of the endopodites accompanied by a loss of spinning tubes, the last step being the suppression of the colulus and the complete approximation of the spinnerets.

Embryologically the cribellum appears comparatively early and is present in spiderlings before they leave the cocoon. Similarly the colulus is developed before even the traces of abdominal segmentation have disappeared. To give an idea of the changes which take place in a spider between the completion of its embryological development in the egg and maturity, I shall give here a brief description of a spiderling of *Lycosa helluo* taken at random from a cocoon and let the reader compare it with a specimen of a mature spider of the same species or at least with a description of it as given by Chamberlin in his excellent monograph of North American Lycosidae.

At this stage the spiderling has neither eyes, nor hair, nor spines. Its body and appendages are smooth and colorless. The shape of the carapace is approximately the same as that of the mature spider, though I have not subjected it to careful measurements. The sternum is oval, but is transversely wider than long. The fang of the chelicerae is the only chitinized structure. It is extraordinarily small, as wide at base as long, and about as long as one-half the diameter of the basal article of the chelicerae. The maxillary plates are clearly defined, but do not have the shape as in mature spiders. The colulus and the spinnerets are very well developed. The anterior spinnerets sit somewhat in advance of the other spinnerets. The anal tubercle is quite prominent. It is oval and above it the dorsal intersomitic lines are still in evidence. Both lung-slits are in their proper positions and the rudiments of lungs already discernible. The tracheal spiracle is clearly visible in front of the spinnerets and the bases of the two tracheal stems are also discernible. There are as yet no traces of the genital rima. The segments of the legs are clearly defined, but do not have the final proportion. The trochanteres are still without a notch. There are three claws on each tarsus, but the claws are quite smooth.

If we now take a spiderling of the same species from the mother's back we find already some important changes. There is as yet no pubescence of any kind, but pigmentation has appeared on the carapace. The eyes are present, but their arrangement is that of a Pisaurid and not of a Lycosid. The sternum is now narrower, but is still wider than in the

adult. Intersomitic lines can still be discerned above the anal tubercle. Pubescence appears after the first molt. The margins of the chelicerae and the spines on legs assume, however, their final structure only after three more molts.

Taking all the evidence into consideration, we may assume, therefore, that the ancestors of Arachnomorph spiders all possessed a cribellum and had three claws on their tarsi. The latter is evidenced by the fact that spiderlings of two-clawed species possess in their early stages three claws, and that a rudimentary claw is still present on the anterior tarsi of some Zoropsids. The calamistrum, on the other hand, must have been a somewhat later acquisition and, once acquired, persisted until the present day. It disappears only in the males of various families after the last molt, being clearly and perfectly defined in all earlier stages. It also becomes more irregular in the two-clawed Zoropsidae and in the Tengellidae—a condition evidently subsequent to its development and leading to its final disappearance.

The similarity in structure and habits of Cribellate and Ecribellate spiders may be readily understood from this point of view. It is not convergence, nor is it spontaneous development of specialized yet identical structures in phylogenetically different families. *It is the development of ecribellated families from cribellated ones through the fusion and transformation of the cribellum into a colulus.* Rather than imagine the separation of cribellated spiders from ecribellated ones and the subsequent independent development of the two divisions, *we have before us a complicated process by which at different times both new cribellated and ecribellated families were produced from the original three-clawed cribellated ones.* It is safer not to unite cribellated and ecribellated forms under a single family, as Dahl did it in the case of the Urocteidae, even though it seems quite reasonable to consider the latter a younger branch of the Oecobiidae. One may be asked, why, then, not segregate the cribellated families under a single division of Cribellatae, as Simon does it? But such a segregation would imply the separation of the Arachnomorph spiders at an early date into the two divisions, whereas my contention is that no such separation took place at one single time. Moreover, the process of evolution must have embraced ecribellated families which in their turn gave rise to new ecribellated families.

The presence of a calamistrum in spiders with a cribellum does not present any great difficulties. It represents nothing but modified hair and varies in length and arrangement. Such spiders as some Zoropsids with a more or less irregular calamistrum give us a clue to its origin. In the cribellated ancestral stock from which Tertiary and Recent Arach-

nomorph spiders developed, the cribellum may have had simply the shape of an indefinite stretch of somewhat modified hair. Gradually the irregularity gave place to a single or double row of specialized hair, while in forms which lost the cribellum, the calamistrum, instead of developing, completely disappeared.

The organs of respiration present a greater difficulty. We know that the tracheal system has developed from the book-lungs and is of a more recent date. Besides the Hypochilidae with two pairs of lungs, we have the Dysderidae and Oonopidae with one pair of lungs and one pair of tracheal spiracles; we have the Caponiidae and Telemidae without lungs, but with two pairs of tracheal spiracles and we have the bulk of spiders with one pair of lungs and a single tracheal spiracle. The single median spiracle undoubtedly developed from a fusion of a pair of spiracles, first through the formation of a transverse common groove uniting the two spiracles and serving as a sort of a shallow atrium, and then through an approximation of the spiracles, together with a shortening and deepening of the uniting groove. The first step in this direction may still be observed in the Filistatidae. The somitic position of the spiracle remains always the same. Its distance from the lungs is of secondary importance and is entirely dependent upon the growth of the third abdominal somite. The position of the spiracle both in the Argyronetidae and Anyphaenidae considerably in advance of the spinnerets must therefore be regarded as the older one.

The transformation of the first pair of lungs into tracheal tubes must be much more recent. We may imagine such a transformation taking place without considerable modification of other characters. Nevertheless, I cannot agree with Fage when he waves aside this difficulty and unites the Telemidae in one family with the Leptonetidae, while at the same time separating from them the Ochyroceratidae. The latter have scarcely any important structural difference from the Leptonetidae and should form a sub-family of these, while the Telemidae undoubtedly deserve the rank of a family.

The position of the eyes and their number are of considerable interest. Since it has been established that both may vary within the same family and even genus, their value as a character has been considerably diminished. Moreover, the arrangement of the eyes has been studied entirely by systematicians and not by morphologists. The direct eyes, usually recognizable from the others without microscopic dissection, have been designated as the anterior median eyes. In the majority of cases these eyes, together with a pair of indirect eyes, form a slightly recurved or procurved line which is spoken of as the first row of eyes, while the other

two pairs of the indirect eyes form the second row. From this supposedly primitive position, the other arrangements of the eyes are derived, such as in Lycosidae, Attidae, Ctenidae, etc. The process by which such an arrangement has been arrived at is, however, practically disregarded, often misunderstood and misinterpreted, as I shall presently show.

The original number of eyes in spiders seems to be eight. Of these the pair of direct eyes belongs to the first cephalic somite, the three pairs of indirect eyes to the second cephalic somite. As I shall describe in another paper, the optic ganglion for the indirect eyes of each side is distinctly composed of three lobes, each furnishing a nerve to its eye. These lobes have a dorso-ventral sequence in the optic ganglion. The dorsal lobe innervates the so-called anterior lateral eye, the median lobe the posterior median eye, and the ventral lobe the posterior lateral eye. Thus we have in reality a first, second and third indirect eye on each side. An examination of the eyes of various spiders leads me to believe that there may have been more than one original plan for the indirect eyes. One is found in *Blechroscelis* and consists of an incurved row of contiguous eyes on each side; another in *Dolomedès,* with an excurved row. From the former type all groups of eyes in which the so-called posterior row is procurved may be derived without much difficulty. Here, then, the third indirect eyes form the posterior median pair. We find this arrangement, with many variations, in *Caponia, Prodidomus, Peucetia,* and still further modified in *Idiops, Epeira, Dysdera.* In the second type the so-called posterior median eyes are the second indirect eyes. This happens in all spiders with a recurved posterior row, as in *Dolomedes, Lycosa, Phidippus, Eriodon, Eresus.* A glance at the accompanying diagrams will aid in an understanding of the difference. From a morphological and phylogenetic point of view it would be much more correct to speak of an incurved and excurved row of indirect eyes of each side than of a procurved and recurved row of anterior and posterior eyes, as is done by all systematists. Yet, in the absence of any research in this field, we have for the present to follow the generally accepted method.

Since our knowledge of the comparative value and historical sequence of the most important characters is so imperfect we shall be justified in treating the subject of phylogeny in spider families with the utmost caution. The accompanying phylogenetic tree is therefore nothing but the first attempt to embody some of the aforesaid conclusions and observations into a diagrammatic presentation of the subject. Instead of two lines of development with astonishing convergence, we may accept at least three, if not more, independent lines of progressive developmnt,

with occasional simplification or disappearance of structures functionally become useless.

One of these evolutionary lines would be chiefly characterized by the primitiveness and comparative instability of the respiratory system, peculiarities in the structure of the mouthparts and arrangement of eyes, and may be considered as represented by the following families: Hypochilidae, Filistatidae, Sicariidae, Dysderidae, Oonopidae, Ammoxenidae, Oecobiidae, Urocteidae, Caponiidae, Prodidomidae, Leptonetidae and Telemidae. I have already discussed the case of the Telemidae and Leptonetidae. The Oonopidae are placed by Dahl as a sub-family of the Dysderidae and the Leptonetidae as a sub-family of the Sicariidae. Both have, in my judgment, sufficiently important characters to warrant their separation or, perhaps more correctly, their retention in separate families. Simon establishes a special family, Hadrotarsidae, for Thorell's *Hadrotarsus* and Keyserling's *Gmogala*. I have no first-hand knowledge of these spiders, and Simon's characterization is also based on the original descriptions of the authors mentioned. These descriptions are entirely inadequate for a clear separation of the Hadrotarsidae from the Oonopidae. It is possible that the two genera belong in the family Oonopidae, in the sub-family Gamasomorphinae, or in a special sub-family. Only a re-examination of specimens could decide this point.

The second line of development is represented by spiders with two claws and ungual tufts. I refer to this group the Zoropsidae, Acanthoctenidae, Ctenidae, Drassidae (Gnaphosidae), Homalonychidae, Sparassidae (Heteropodidae), Selenopidae, Platoridae, Thomisidae, Aphanthochilidae, Clubionidae, Attidae and the Tertiary family Parattidae. The Tengellidae may be regarded as a small separate line closely connected with the Zoropsidae. The scope of each family has to be considered in detail.

The family Zoropsidae, as I delimit it here, corresponds to Simon's sub-family Zoropsinae. Dahl found it necessary to segregate all genera except *Zoropsis* under a family Zorocratidae in a different sub-order from the Zoropsidae. His logic is not clear to me. In this case he bases the separation of his sub-orders on the curvature of the posterior row of eyes. But, as I have shown above, the phylogeny of this row is as yet not clearly understood. Simon places the Ctenidae as a sub-family of the Clubionidae, while Dahl not only separates them as a distinct family, but goes so far as to place in the same family the Acanthoctenidae as a sub-family. That the Acanthoctenidae, except for the cribellum and calamistrum, have more in common with the Ctenidae than the latter with the Clubionidae, cannot be denied. At the same time in this, as

in other cases, I think it advisable to separate cribellated from ecribellated forms, considering the latter as a later development of the former. To finish with Simon's Clubionidae, I may state that I limit this family now to his Clubioninae, Liocraninae, Micariinae and Corinninae, while the Selenopinae and Sparassinae seem to have sufficiently distinct characters in the laterigradeness of their legs, in the structure of the eyes in the former and in the structure of the metatarsi in the latter, to elevate them to the rank of distinct families.

Simon's Drassidae seem to be an incongruous group. Following the example of other arachnologists, I retain in this family only spiders with widely separated anterior spinnerets—that is, Simon's Hemicloeinae and Drassodinae. His Cithaeroninae seem to me to belong to the family Zodariidae, unless we limit the Zodariidae to three-clawed species, while his Cybaeodinae, with which I am not acquainted from first-hand knowledge, are, in my opinion, a heterogeneous group, to be provisionally referred to the Clubionidae.

Simon places the Homalonychidae as a sub-family of the Zodariidae, but they have so little in common that a separation is absolutely necessary. They seem to be in every respect much nearer the Sicariids than the Zodariids.

The Aphanthochilidae have been already separated by other arachnologists, and I think that there is quite sufficient reason for doing it.

The Parattidae is a family which I have established for Tertiary spiders with an arrangement of eyes unknown in other families. Unfortunately, other important characters are missing and the characterization of this family from an arachnological point of view is very unsatisfactory.

The Attidae (Salticidae) represent a very large and uniform family. So far, they have defied all attempts at a reasonable subdivision into smaller groups. Simon's three divisions based on the number and structure of teeth on the margins in chelicerae are entirely artificial, but no one has offered anything better. Dahl mentions under Salticidae fourteen sub-families, but gives no distinctive characters.

Dahl's attempt to elevate all the sub-families of Thomisidae to the rank of families is not commendable. The family is a very uniform one and, with the exception of the Aphanthochilidae, I accept in full Simon's sub-families.

The third evolutionary line embraces the remaining twenty families and represents four distinct branches of mostly three-clawed spiders. One of these branches I imagine to be composed of Eresidae, Zodariidae, Palpimanidae, Pisauridae, Lycosidae, Senoculidae and Oxyopidae. Of these the Zodariidae and Palpimanidae present the greatest difficulties.

Both possess forms with three and two claws. We can understand the presence of such forms among three-clawed ones as a further step in the evolution due to change of habits. The Palpimanidae I limit to the two sub-families having only two spinnerets, the Palpimaninae and the Stenochilinae, and I add a third sub-family, Hermippinae, composed of the genera *Hermippus, Lutica* and *Mallinus.* I do so because I believe that the disappearance of the spinnerets representing the second pair of pleiopods is a much more remarkable character than the structure of the scopula or the presence or absence of a serrula on the chelicerae. We meet with a similar condition again in the Archaeidae, but in no other Arachnomorph spiders, while in the Mygalomorph *Diplothele* and *Anisaspis* the remaining two spinnerets are the posterior ones.

The family Zodariidae thus limited still includes forms not entirely congruous. It contains six sub-families, the Cydrelinae, Cryptothelinae, Zodariinae, Huttoninae, Cithaeroninae and Storenomorphinae.

The separation of the Lycosidae from the Pisauridae is not an easy one, as they represent undoubtedly a later offshoot of the same stock. This is evidenced by the gradual change in the arrangement of the eyegroup during the growth of the spiderling and by the higher development of maternal instincts. The affiliation of the sub-families Rhoicininae and Hippaseinae is not clear to me and I leave them, therefore, provisionally in the family Lycosidae, following Simon's classification.

The second branch of the third evolutionary line may be delimited as including the families Amaurobiidae, Psechridae, Agelenidae and Hersiliidae. The first three are undoubtedly closely related to each other. The Hersiliidae represent such highly specialized forms that one is at a loss in the search for their nearest relatives. They have a certain similarity in the structure of the spinnerets with the Agelenidae. Their habits are arboreal or in other cases comparable to the habits of *Coelotes* and *Amaurobius.* Their eyes have a peculiar arrangement permitting them to see objects moving on their flanks but not above or in front of them, with the result that it is easy to catch them by placing a finger right over their carapace, in the axis of their body, while they make off with great rapidity if one approaches them from the side.

I prefer to limit the family Psechridae to the Psechrinae, Stiphidiinae and Matachiinae of Dalmas, and to remove the genus Themacrys, referred by him to this family, by placing it under the Tengellidae, on account of the presence of scopulae on their tarsi.

The family Amaurobiidae shows more relationship to the Agelenidae than to the Dictynidae, under which Simon has placed them. Their tarsi are with numerous trichobothria arranged in a row and their habits are very much like those of *Coelotes.*

The Agelenidae are well delimited by Simon and I do not see any reason why Dahl finds it necessary again to place the *Nicodamini* in the family Theridiidae, as had been done by Keyserling at a time when the characters of that family were less understood than at present.

The third branch would include the families Dictynidae, Pholcidae, Theridiidae, Linyphiidae and Archaeidae. As already stated, I have removed the Amaurobiidae from the Dictynidae, leaving in the latter the more closely allied forms. At the same time I add to the family the genus *Aebutina,* placed by Simon under Uloboridae. I think that in this case the structure of the eyes and of the cribellum are of more importance than the presence of serrated bristles, because they represent older characters. The Theridiidae comprise all spiders with a comb on the fourth tarsus. It is a very well delimited family and consequently extremely difficult of subdivision. The Archaeidae belong mostly to the Amber, with but two genera surviving. The name is an unfortunate misnomer, for these spiders represent an extreme stage of development, in which the chelicerae became modified and placed considerably in front of the mouth. We find a similar condition in some recent Dictynas and Linyphias, another indication that these families have many genetic similarities.

The fourth and last branch of the third line would be composed of the families Uloboridae, Dinopidae, Argiopidae and Mimetidae. Of these I restrict the Uloboridae to the sub-families Uloborinae and Miagrammopinae distinguished by the structure of the sternum. The genus *Hyptiotes* falls, therefore, within the Uloborinae and not Miagrammopinae, as Simon has it. The family Argiopidae is, perhaps, even with these limitations (Simon includes under Argiopidae also the Linyphiidae), still too large. Nevertheless, in the absence of recent monographic treatment of the family, it seems to be wisest not to split it. I recognize the sub-families Tetragnathinae, Metinae, Nephilinae, Argiopinae, Araneinae, Gasteracanthinae and Theridiosomatinae. Of these the Argiopinae correspond to Simon's Argiopeae, Gasteracanthinae to Simon's Gasteracantheae, Micrathenae and Xylethreae and the genus *Tecmessa,* while the Araneinae comprise all the other groups.

The thoughts which guided me in the genetic redistribution of families cannot find their expression in the key to the families of spiders which I add here for the convenience of arachnologists. It would complicate matters if I were to try to embody them and to follow lines of development and not present morphological characters. The key should, therefore, be regarded as an artificial one. Nevertheless, the characters have been chosen with great care, and besides distinctive characters the most

important additional characters are mentioned wherever they have not been used already for the alternatives.

Order ARANEAE

Characters of the Order. Arachnida in which all thoracic somites are permanently fused with the cephalic somites, while the abdomen either remains segmented or more commonly loses its external segmentation during the embryological development. The abdomen is joined to the cephalothorax by a thin petiolus (first abdominal somite) and bears spinnerets (modified pleiopods) on the fourth and fifth somites. Cephalic and thoracic tergites form a single carapace in which the two regions are usually delimited by a groove. Cephalic sternites wanting, thoracic sternites form a single sternum. Eyes simple, never more than eight in number. The first pair belonging to the first cephalic somite alone have a direct retina.[2] All the other eyes belong to the second cephalic somite and have an indirect retina. Reduction in the number of eyes usually begins with the first pair. Chelicerae two-jointed, retrovert. Pedipalpi six-jointed, pediform. Maxillary lobes, if present, only on the coxae of the pedipalpi and harboring within them maxillary glands. Legs typically seven-jointed, with two or three claws. Lyriform organs of unknown function present on all appendages. Respiration aerial, by means of book-lungs or tracheal tubes and book-lungs, with openings on the ventral surface of the second and third abdominal somites. Digestive system with pumping pharynx, thin esophagus and pumping gizzard. The midgut characterized by the ramified caeca in the cephalothorax and the presence of a large digestive gland (liver) with several pairs of ducts in the abdomen. Hindgut receives malpighian tubules. Anus always terminal. Modified nephridia in the shape of two coxal glands in the cephalothorax with one or two pairs of openings to the outside. Heart normally with three or four pairs of ostia in the abdomen. Cardio-aortic valve typically situated in the region between the last thoracic and first abdominal somites. Arterial system typical except for a reduction in the neural arteries due to the concentration of the central nervous system. The latter situated entirely in the cephalothorax and composed of a brain and a thoracic ganglionic mass. Original neuromeres recognizable even in the adult. Two poison glands in the cephalothorax with ducts opening near the tip of the fangs. Spinning glands in the abdomen opening by means of spinning tubes on the spinnerets. Never

[2] Since the completion of my paper on Tertiary spiders I had opportunity to examine quite young spiderlings and am positive that the direct eyes belong to the first cephalic somite. The results of this observation will be published in the near future.

more than eight, nor less than two spinnerets. Those derived from exopodites are two-, three- or four-jointed, those from endopodites single-jointed. Sexes separate. Reproductive glands in the abdomen. Genital opening in both sexes on the ventral surface of the second abdominal somite. A pair of additional openings are often present in the female leading into the sperm receptacles. The genital openings of the female often with a chitinous epigynum. Male copulatory organs on the terminal joint of the pedipalpi. All species oviparous without exception.

Key to Sub-Orders, Families and Sub-Families

1. Abdomen segmented. Not more than two posterior abdominal somites lost. Anal tubercle separated by a considerable space from the spinnerets. Chelicerae so articulated that fangs move up and down. Maxillary lobes wanting. Lungs 4.

Sub-Order LIPHISTIOMORPHAE

a. Recent. Thoracic groove transverse. Eyes 8, heterogeneous, in a single group. Sternum very long and narrow. Chelicerae without rastellum. Lip free. Maxillary lobes completely wanting. Legs with spines, without scopulae or claw-tufts. Three claws. Upper claws similar, with 2 or 3 teeth in a single row..... Family LIPHISTIIDAE.

* Carboniferous ..... ?

b. Eyes on a tubercle, in a compact group..... Family ARTHROLYCOSIDAE.[3]

* Eyes in two rows. Claws 2, smooth..... Family ARTHROMYGALIDAE.[4]

* Abdomen entire. Anal tubercle situated immediately behind the spinnerets. Never more than six spinnerets..... 2

2. Chelicerae so articulated that the fangs move up and down. Two pairs of lungs. Posterior spinnerets 3 or 4 jointed. Heart with four pairs of ostia. Coxal glands with two pairs of openings, one behind the first coxae, the other behind the third coxae. Sub-Order MYGALOMORPHAE..... 3

* Chelicerae so articulated that the fangs move in and out. Usually one pair of lungs and a single tracheal spiracle, with

[3] Including the genus *Arthrolycosa* with two American species, *A. antiqua* and *A. danielsi*. Possibly also the genera *Protolycosa* and *Eocteniza*.

[4] Including the new genus *Arthromygale*, with the species *A. carbonaria*, *A. fortis* and *A. beecheri*, and the genus *Rakovnicia*. Possibly also the genera *Geralycosa* and *Perneria* and the species *Arthrolycosa lorenzi* and *Arthrolycosa palaranea*.

some exceptions. Posterior spinnerets only two-jointed. Heart with three, rarely with two, pairs of ostia. Coxal glands with a single pair of openings situated behind the first pair of coxae. Sub-Order ARACHNOMORPHAE..... 10

Sub-Order MYGALOMORPHAE

3. All tarsi with a scopula. Two claws..... 4

* At least third and fourth tarsi without scopula. Usually three claws, sometimes two. Claw-tufts wanting..... 6

4. Claw-tufts wanting. Claws with two rows of teeth. Lip immobile. Chelicerae without rastellum. Thoracic groove transverse. Eyes 8, heterogeneous, in a compact group. Maxillary lobes wanting. Spinnerets 4, anterior pair small, approximated, posterior pair heavier and longer, with short terminal joint.....
Family PYCNOTHELIDAE.

* Claw-tufts present. Claws with a single row of teeth or smooth. Lip free 5

5. Last joint of posterior spinnerets very short. Spinnerets 4 or 2. Thoracic groove longitudinal. Eyes 8, heterogeneous, variously arranged. Chelicerae usually with a rastellum. Maxillary lobes wanting. Claws similar, with few teeth in a single row or quite smooth.....Family BARYCHELIDAE.

* Last joint of posterior spinnerets at least as long as the preceding one. Spinnerets 4. Thoracic groove transverse. Eyes 8, heterogeneous, in a compact group. Chelicerae without rastellum, sometimes with a stridulating organ. Maxillary lobes wanting. Claws similar, pectinate in a single row.....Family THERAPHOSIDAE.[5]

6. Chelicerae with a rastellum. Maxillary lobes wanting. Spinnerets 4, rarely 6, rather short. Anterior spinnerets more or less approximated. Thoracic groove variable. Eyes 8, heterogeneous, variously arranged. Three claws. Upper claws similar, pectinate...
Family CTENIZIDAE.

a. Lip immobile. Sternum with six sigilla. Thoracic groove transverse. Spinnerets 4. Upper claws with a single row of teeth....
Sub-Family Actinopodinae.

* Lip free..... *b*

b. Thoracic groove transverse. Sternum with 2 or 4 sigilla. Spinnerets 4. Upper claws either with a single or a double row of teeth. First and second tarsi sometimes with a light scopula....
Sub-Family Ctenizinae.

* Thoracic groove longitudinal. Sternum with 4 small sigilla. Upper claws with a single row of teeth. Spinnerets 4 or 6.....
Sub-Family Brachybothriinae.

* Chelicerae without a rastellum..... 7

[5] Synonym AVICULARIINAE of Simon.

7. Lip free. Maxillary lobes wanting. Spinnerets 4 or 6. Posterior spinnerets very long. Claws 3, upper claws with a single or double row of teeth. Eyes 8 (or 6), heterogeneous, variously arranged. Web-weavers.....Family DIPLURIDAE.

a. Sternum very long and narrow, without sigilla. Thoracic groove transverse. Eyes 8, in two rows. Spinnerets 4, anterior spinnerets approximated. First and second metatarsi and tarsi with a scopula.....Sub-Family DOLICHOSTERNINAE.

* Sternum normal. Anterior spinnerets wide apart. No scopulae on legs ..... *b*

b. Sternum with 6 small submarginal sigilla. Spinnerets 4 or 6. Eyes 8 (or 6), in a compact group. Thoracic groove transverse.....
Sub-Family DIPLURINAE.

* Sternum without sigilla. Spinnerets 6. Eyes 8, in two rows. Thoracic groove longitudinal.....Sub-Family HEXURINAE.

* Lip immobile..... 8

8. Maxillary lobes well developed. Six spinnerets. Anterior spinnerets approximated. Thoracic groove longitudinal or wanting. Sternum with 8 small submarginal sigilla. Three claws. Upper claws pectinate in a single row. Eyes 8, heterogeneous, arranged in three groups.....Family ATYPIDAE.

* Maxillary lobes wanting or only rudimentary. Not more than 4 spinnerets 9

9. Three claws. Upper claws with one or several teeth, sometimes dissimilar. Spinnerets 4. Thoracic groove transverse. Sternum normal, with sigilla. Eyes 8, heterogeneous, in two rows. Maxillary lobes wanting. Hair simple.....Family MIGIDAE.

* Two claws with a single tooth each. Sometimes rudimentary third claw present. Spinnerets 4 or 2. Thoracic groove transverse. Sternum wider than long, without sigilla. Eyes 8, heterogeneous, in a compact group. Coxa of pedipalpi with strongly produced anterior inner angle, forming a rudimentary maxillary lobe. Body covered with scales and claviform hair.....
Family PARATROPIDIDAE.

SUB-ORDER ARACHNOMORPHAE

10. Cribellum and calamistrum present[6]..... 11

* Cribellum and calamistrum wanting..... 22

11. Four lungs. Cribellum entire. Spinnerets 6. Thoracic groove longitudinal. Lip free. Maxillary lobes divergent, with scopula. Chelicerae without boss, both margins with several teeth. Tarsi without claw-tufts or scopula. Claws 3. Upper claws similar, pectinate in a single row. Eyes 8, heterogeneous, in two rows.

[6] Except in the case of some mature males in which these structures are rudimentary.

Anal tubercle normal. Male copulatory apparatus terminal. Web meshed.....Family HYPOCHILIDAE.

* Two lungs and a single tracheal spiracle..... 12

12. Tarsi with two claws..... 13

* Tarsi with three claws. (Third claw on the first pair of legs sometimes rudimentary)..... 14

13. First and second tarsi and metatarsi always with a scopula. Eyes 8, homogeneous, diurnal, in two rows. Cribellum divided. Spinnerets 6. Thoracic groove longitudinal. Lip free. Maxillary lobes with scopula. Chelicerae with boss, both margines with teeth. Tarsal trichobothria irregular, forming two or more rows. Claw-tufts wanting. Claws pectinate in a single row, similar... Family ZOROPSIDAE.[7]

* None of the tarsi or metatarsi with a scopula. Eyes 8, homogeneous, diurnal, in three rows, first row composed of the two direct eyes, second of the two anterior lateral and 2 posterior median eyes, third of the two posterior lateral eyes. Cribellum either divided or entire. Spinnerets 6. Thoracic groove longitudinal. Lip free. Maxillary lobes with scopula. Chelicerae with boss, both margins with teeth. Tarsal trichobothria irregular, tarsi with claw-tufts. Claws pectinate in a single row, similar..... Family ACANTHOCTENIDAE.

14. First and second tarsi and metatarsi with scopula. Two rows of trichobothria on tarsi. Cribellum either divided or entire. Spinnerets 6. Calamistrum with different stages of irregularity. Eyes 8, in two rows. Chelicerae with teeth on both margins. Maxillary lobes slightly converging, with scopula. Upper claws pectinate in a single row, similar.....Family TENGELLIDAE.[8]

* None of the tarsi or metatarsi with a scopula. Tarsi without trichobothria or with a single row of trichobothria..... 15

15. Tarsi with claw-tufts and a single row of trichobothria. Upper claws pectinate in a single row, similar. Cribellum divided or entire. Spinnerets 6. Thoracic groove longitudinal. Eyes 8, homogeneous, diurnal, in two rows. Lip free. Chelicerae with boss, both margins with teeth. Maxillary lobes with scopula..... Family PSECHRIDAE.[9]

* Tarsi without claw-tufts..... 16

16. Lip immobile. Chelicerae without boss, soldered at their base, without scopula. Tracheal spiracle in the shape of a transverse slit considerably in front of the spinnerets. Cribellum divided. Spinnerets 6. Anal tubercle normal. Thoracic groove longitudinal. Eyes 8, heterogeneous, in a compact group. Both margins of

[7] Synonym: ZOROPSINAE of Simon, or Zoropsidae + Zorocratidae of Dahl.

[8] Includes the genera *Tengella*, *Calamistrula* and possibly *Mnesitheus*; also the genus *Themacrys*, referred by Dalmas to PSECHRIDAE.

[9] Includes only the PSECHRINAE, STIPHIDIINAE and MATACHIINAE of Dalmas.

chelicerae smooth. Maxillary lobes converging, without a scopula. Tarsi without trichobothria, metatarsi with from one to several trichobothria in a single row. Upper claws pectinate in a single row, similar. Male copulatory apparatus terminal.....
Family FILISTATIDAE.

* Lip free. Tracheal spiracle close to spinnerets..... 17

17. Anal tubercle large, two-jointed, with a fringe of long hair. Terminal joint of posterior spinnerets long, with spinning tubes along inner edge. Spinnerets 6. Cribellum divided. Carapace without thoracic groove. Eyes 8, heterogeneous, in a compact group. Sternum wider than long. Chelicerae without boss or scopula. Both margins smooth. Maxillary lobes strongly converging, without scopula. Legs without spines. Tarsi without trichobothria. Metatarsi with 1 or 2 trichobothria. Upper claws similar, pectinate in a single row.....Family OECOBIIDAE.

* Anal tubercle normal. Terminal joint of posterior spinnerets not unusually long..... 18

18. Eyes heterogeneous. Anterior median eyes alone diurnal..... 19

* Eyes homogeneous, all diurnal..... 20

19. Tarsi with a single row of trichobothria. Cribbellum divided. Spinnerets 6. Thoracic groove longitudinal. Eyes 8, in two rows. Chelicerae with boss and scopula, both margins with teeth. Maxillary lobes more or less parallel, with scopula. Upper claws pectinate in a single row, similar....Family AMAUROBIIDAE.[10]

* Tarsi without trichobothria. Cribellum usually entire, sometimes divided. Spinnerets 6. Thoracic groove longitudinal. Eyes 8 (or 6), in two rows. Chelicerae with boss and scopula, both margins with teeth. Maxillary lobes converging, with scopula. Upper claws pectinate in a single row, similar..Family DICTYNIDAE.[11]

20. Cribellum divided. Posterior spinnerets longer and heavier than anterior ones. Spinnerets 6. Chelicerae with boss and scopula, both margins with teeth. Eyes 8, in three rows, first row of four eyes. Maxillary lobes converging, with scopula. Tarsi and femora without trichobothria, metatarsi with 1 or 2 trichobothria. Upper claws pectinate in a single row, dissimilar, not accompanied by serrated bristles.....Family ERESIDAE.[12]

* Cribellum entire. Spinnerets more equal. Claws similar, accompanied by serrated bristles..... 21

21. Femora without trichobothria. Metatarsi with 1 or 2 trichobothria, tarsi without trichobothria. Spinnerets 6. Eyes 8, in three or two rows, first row of four eyes. Maxillary lobes slightly divergent. Upper claws pectinate in a single row.....
Family DINOPIDAE.

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[10] Includes the genera *Amaurobius, Badumna, Nurscia, Callevа, Auximus, Taurongia, Callevopsis, Aphyctoschaema, Aymarella, Ixeuticus.*

[11] Including the genera *Dictyna, Lathys, Protadia* etc. and also *Aebutina.*

[12] Including also the genus *Penestomus.*

* Femora with trichobothria. Metatarsi with 1 or 2 trichobothria. Tarsi without trichobothria. Spinnerets 6. Eyes 8, in two rows. Maxillary lobes more or less parallel. Upper claws pectinate in a single row.....Family ULOBORIDAE.

a. Sternum normal.....Sub-Family ULOBORINAE.[13]

* Sternum with strong lateral projections between second and third coxae.....Sub-Family MIAGRAMMOPINAE.

22. Four spiracles, the first pair belonging either to book-lungs or to tracheal tubes, the second pair always to tracheal tubes..... 23

* Three spiracles, the pair of anterior spiracles belonging to book-lungs, the third median spiracle to the common stem of tracheal tubes. This spiracle is usually situated immediately in front of the spinnerets except in few cases when it is considerably in front of the spinnerets 26

23. The first pair of spiracles belongs to book-lungs..... 24

* The first pair of spiracles belongs to tracheal tubes. Book-lungs entirely absent..... 25

24. Claws pectinate in a single row. Thoracic groove wanting. Chelicerae without boss, with long oblique margins. Lip free, long. Maxillary lobes parallel, with a scopula. Colulus present. Spinnerets 6, anterior pair approximated. Eyes 6 (or 0), direct eyes always wanting. Posterior coxae separated by the sternum. Tarsi without trichobothria. Claws three or two. Pedipalp of female with claw. Heart with only 2 pairs of ostia..Family DYSDERIDAE.

a. Eyes in three groups of two each. Sternum separated from carapace by soft membrane. Tarsi with three claws.....
Sub-Family SEGESTRIINAE.

* Eyes forming a transverse oval. Sternum connected to carapace by hard chitin. Three or two claws.....Sub-Family DYSDERINAE.

* Claws pectinate in two rows. Thoracic groove wanting. Chelicerae without boss, with long oblique margins. Lip free, long or wide. Maxillary lobes converging, with scopula. Colulus absent. Spinnerets 6, anterior spinnerets approximated. Eyes 6 or 0, (compare footnote), direct eyes wanting. Posterior coxae separated by sternum. Tarsi without trichobothria or scopulae. Claws 2. Pedipalp of female without claw.....Family OONOPIDAE.

a. Abdomen soft.....Sub-Family OONOPINAE.

* Abdomen with hard shields.....Sub-Family GAMASOMORPHINAE.[14]

25. Claws three, dissimilar, pectinate in a single row. Chelicerae without boss, their oblique margins with teeth. Lip immobile. Colulus present. Spinnerets 6, anterior pair longest. Eyes absent.....
Family TELEMIDAE.

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[13] Including *Uloborus*, *Sybota* and *Hyptiotes*.

[14] Simon's family HADROTARSIDAE belongs in this vicinity. Perhaps the genera *Hadrotarsus* and *Gmogala* should form a separate sub-family characterized by the presence of 8 eyes, four spinnerets and wide lip.

* Claws three or two, similar, pectinate in a single row. Chelicerae without boss, their margins smooth. Lip free. Colulus wanting. Spinnerets 6, posterior pair longest. Eyes 8 or 2, in all cases direct eyes present.....Family CAPONIIDAE.

26. Spinnerets 2, the median and posterior spinnerets wanting. Lip free, normally long. Colulus wanting. Chelicerae with boss and smooth, straight margins. Maxillary lobes converging, with scopula and serrula. Pedipalp of female without claw. Posterior coxae separated by sternum. Anterior tarsi and metatarsi with a thick scopula on inner edge only. Claw-tufts wanting. Claws three or two, dissimilar, pectinate in a single row. Eyes 8, heterogeneous.....Family PALPIMANIDAE.

a. Anterior tarsi club-shaped, heavier toward the end than at the base. Sub-Family PALPIMANINAE.

* Anterior tarsi normal..... *b*

b. Anterior tarsi and metatarsi with inner scopula..... Sub-Family STENOCHILINAE.

* Anterior tarsi and metatarsi without scopula..... Sub-Family HERMIPPINAE.[15]

* Spinnerets 6, or if only two then the chelicerae considerably removed from the mouth..... 27

27. Tarsi with two claws..... 28

* Tarsi with three claws, rarely third claw rudimentary..... 40

28. Tertiary spiders. Eyes 8, in two rows, anterior row composed of very large, round and nearly equidistant eyes. Posterior row of four very small eyes, the posterior median situated between and scarcely behind the anterior median ones. Family PARATTIDAE.

* Recent and extinct spiders. Arrangement of eyes never such as described above..... 29

29. Chelicerae soldered at base, with boss and a row of teeth along their entire inner edge. Lip immobile. Maxillary lobes converging, with scopula. Colulus absent. Spinnerets 6, anterior pair wide apart, posterior pair longest. Posterior coxae separated by sternum. Trochanteres notched. Tarsal trichobothria numerous. Scopulae and claw tufts wanting. Claws similar, pectinate in a single row. Eyes 8, in a compact group..... Family AMMOXENIDAE.

* Chelicerae and lip free..... 30

30. Eyes in three rows, first row composed of four eyes, second and third of two eyes each. Direct eyes by far the largest, eyes of second row the smallest. In some cases anterior row so strongly recurved, that eyes appear in four rows. All eyes diurnal. Colulus wanting. Spinnerets 6, anterior pair approximated, heavier than the others. Chelicerae without boss. Maxillary lobes more or

[15] Includes the genera *Hermippus*, *Lutica* and *Mallinus*.

less parallel. Pedipalp of female without claw. Posterior coxae contiguous. Anterior trochanteres often slightly emarginate. Tarsi with claw-tufts. Claws dissimilar, pectinate in a single row.....Family ATTIDAE.

* Eyes either in two rows, or if in three rows then second row composed of four eyes or eyes distinctly heterogeneous..... 31

31. Sternum wider than long, broadly truncated behind. Body very flat. Thoracic groove wanting. Lip long. Chelicerae with boss, margins oblique, toothed. Maxillary lobes converging. Both anterior and posterior pair of coxae widely separate. Tarsi without scopula or claw-tufts. Claws dissimilar, pectinate in a single row. Colulus wanting. Spinnerets 6, anterior pair longest, wide apart. Median spinnerets situated between the anterior pair, with spinning tubes in two rows. Eyes 8, heterogeneous, in two rows of four each. Legs laterigrade.....Family PLATORIDAE.

* Sternum normal. Median spinnerets not unusual..... 32

32. Eyes 8, heterogeneous, in two rows, first row composed of six eyes. Only the outer eyes of the first row of the nocturnal type. Body flat. Legs laterigrade. Thoracic groove longitudinal. Chelicerae with boss, margins oblique, toothed. Maxillary lobes about parallel, with scopula and serrula. Pedipalp of female with claw. Posterior coxae separated by sternum. Trochanteres sometimes notched. First and second tarsi with scopula. Claws similar, smooth or pectinate in a single row. Colulus wanting. Spinnerets 6. Anterior pair approximated..Family SELENOPIDAE.

* First row of eyes never composed of six eyes..... 33

33. Anterior pair of spinnerets wide apart..... 34

* Anterior pair of spinnerets contiguous or nearly so..... 35

34. Eyes 8, in two rows of four eyes each, direct eyes alone diurnal. (If only six eyes, then direct eyes wanting.) Thoracic groove longitudinal. Chelicerae with boss, with toothed margins. Maxillary lobes converging, with scopula and serrula and with a deep oblique impression across the middle. Posterior coxae separated by sternum. Trochanteres without notch. All tarsi with scopula and claw-tufts. Claws similar, pectinate in a single row or smooth. Colulus wanting. Spinnerets 6. Pedipalp of female with claw.. Family DRASSIDAE.[16]

a. Legs laterigrade. Fourth coxae longest. Median spinnerets sometimes unusually developed.....Sub-Family HEMICLOEINAE.

* Legs prograde. First coxae longest. Median spinnerets never unusually developed.....Sub-Family DRASSODINAE.

* Eyes 8, in a compact group, forming three rows, the first row composed of four eyes. Direct eyes alone diurnal. Thoracic groove longitudinal. Chelicerae without boss, margins oblique, smooth. Maxillary lobes converging. Pedipalp of female without claw.

[16] Synonym GNAPHOSIDAE.

Posterior coxae separated by sternum. Trochanteres without notch. Tarsi without scopula or with light scopula on anterior pair only. Claw-tufts present. Claws similar, smooth. Spinnerets 6.....Family PRODIDOMIDAE.

35. Sternum wide, pointed behind. Posterior coxae far apart. Tarsi with serrated bristles. Thoracic groove deep, longitudinal. Eyes 8, homogeneous, in two rows of four each, posterior row strongly recurved and much wider than anterior row. Chelicerae with boss, without scopula, with transverse, smooth margins. Maxillary lobes converging, with scopula, but without serrula. Pedipalp of female with claw. Claw-tufts present. Claws smooth. Spinnerets 6. Colulus wanting.....Family HOMALONYCHIDAE.

* Sternum oval or long. Posterior coxae approximated. Tarsi without serrated bristles..... 36

36. All tarsi without scopula. Colulus present. Chelicerae with boss, but without scopula, margins usually smooth..... 37

* At least first and second tarsi with scopula. Colulus wanting. Chelicerae with boss and scopula, margins toothed. Thoracic groove longitudinal 38

37. Lip lanceshaped, very long and narrow. Sternum narrow, pointed posteriorly, not reaching beyond third coxae. Fourth metatarsi longer than second. Thoracic groove wanting. Maxillary lobes with scopula and serrula, meeting beyond lip. Pedipalp of female with claw. Claw-tufts present. Claws similar, pectinate in a single row. Eyes 8, homogeneous, in two rows of four each. Legs laterigrade.....Family APHANTHOCHILIDAE.

* Lip not lanceshaped. Sternum normal. Second metatarsi longer than fourth. Maxillary lobes with scopula and serrula. Pedipalp of female with claw. Claw-tufts present in most cases, sometimes absent. Claws similar or dissimilar, pectinate in a single row. Eyes 8, homogeneous, in two rows of four each. Legs laterigrade. Family THOMISIDAE.

a. Lip pointed, long.....Sub-Family STROPHIINAE.

* Lip rounded or truncated at end, shorter..... *b*

b. Tarsi comparatively long and quite broad at base. Claws minute or rudimentary.....Sub-Family STIPHROPODINAE.

* Tarsi and claws normal..... *c*

c. Inferior margin of chelicerae with at least two teeth..... Sub-Family STEPHANOPSINAE.

* Inferior margin of chelicerae smooth..... *d*

d. Integuments with simple hair. Claw-tufts absent or present. First and second pair usually much the longest..... Sub-Family MISUMENINAE.

* Integuments with plumose hair. Claw-tufts always present. Disparity of legs not as great.....Sub-Family PHILODROMINAE.

38. Legs laterigrade. Apical end of metatarsi with a soft trilobate membrane. Eyes 8, homogeneous, in two rows of four each. All tarsi with scopula. Claws similar, pectinate in a single row.....
Family SPARASSIDAE.[17]

* Legs prograde. Apical end of metatarsi with chitinous rim..... 39

39. Eyes 8, in two rows of four each. Scopula of maxillary lobes well delimited, not reaching over external surface.....
Family CLUBIONIDAE.[18]

a. Last joint of posterior spinnerets distinctly conical..... *b*

* Last joint of posterior spinnerets rounded, almost indistinct..... *c*

b. Maxillary lobes narrower in middle. Lip always long.....
Sub-Family CLUBIONINAE.

* Maxillary lobes not narrower in middle than at end.....
Sub-Family LIOCRANINAE.

c. Maxillary lobes subtruncate. Anterior tarsi long. Sternum without a distinct chitinous border.....Sub-Family MICARIINAE.

* Maxillary lobes rounded. Anterior tarsi normal. Sternum with a distinct chitinous border.....Sub-Family CORINNINAE.

* Eyes 8, in three rows, second row of four eyes. Scopula of maxillary lobes extending partly over external surface. Family CTENIDAE.

40. Trochanter of pedipalp inserted on the dorsal surface of the maxillary lobes. Head conspicuously elevated over thorax. Chelicerae inserted far above the mouth, without boss. Upper margin with a series of long, thin spines. Maxillary lobes arched over lip. Tarsi with serrated bristles, but without trichobothria. Claws similar, pectinate in a single row. Colulus present or wanting. Spinnerets 6 or 2. Eyes 8 or 6.....Family ARCHAEIDAE.

* Trochanter of pedipalp inserted on the outer edge of the maxillary lobes. Head not conspicuously elevated. Chelicerae inserted nearer the mouth..... 41

41. Anal tubercle large, two-jointed, with fringe of long hair. Colulus present. Anterior spinnerets approximated, much shorter than posterior spinnerets. Chelicerae soldered at base, without boss. Margins smooth. Maxillary lobes converging, pedipalp of female with claw. Legs without spines. Posterior coxae separated by sternum. Tarsi without serrated bristles or trichobothria. Upper claws similar, pectinate in a single row. Eyes 8, heterogeneous, in two rows of four each.....Family UROCTEIDAE.

* Anal tubercle normal..... 42

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[17] Synonym HETEROPODIDAE.

[18] Family restricted to Simon's CLUBIONINAE, LIOCRANINAE, MICARIINAE, CORINNINAE. The Cybaeodinae (referred to by Simon to the Drassidae and containing but two genera) are placed by me provisionally in the family Clubionidae, but I am unable to give diagnostic characters. From Simon's description I would say that the distinctive characters of the two genera are so important that the sub-family seems to be an incongruous group. Cybaeodes seems to have many characters in common with the Liocranini.

42. First and second tibia and metatarsus with a row of long spines the interspaces between which are occupied by a row of much shorter, curved spines. Chelicerae without boss, with toothed margins. Maxillary lobes converging. Pedipalp of female with claw. Posterior coxae separated. Upper claws similar, pectinate in a single row. Colulus present. Anterior spinnerets approximated. Eyes 8, heterogeneous, in two rows, laterals contiguous.....
Family MIMETIDAE.

* No such arrangement of spines on first and second tibia and metatarsus 43

43. Fourth tarsi with a row of serrated bristles forming a distinct "comb." Chelicerae without boss. Lower margin always smooth, upper margin usually smooth. Legs without spines. Claws similar, pectinate in a single row, or smooth. Colulus present. Anterior spinnerets approximated, heavier than posterior ones. Eyes 8, rarely 6 or 4, heterogeneous, in two rows of four each.....
Family THERIDIIDAE.

* No "comb" on fourth tarsi..... 44

44. Serrated bristles present on tarsi..... 45

* No serrated bristles on tarsi (except in some OXYOPIDAE)..... 49

45. Chelicerae soldered together at base..... 46

* Chelicerae free..... 47

46. Eyes usually six, nocturnal, in three groups of two each, or if eight eyes present, then direct eyes also nocturnal and eyes arranged in two rows of four each. Clypeus high. Chelicerae without boss. Upper margin with a transparent lamina. Lip long. Maxillary lobes converging. Pedipalp in female without claw. Tarsi normal. Upper claws with one or two rows of teeth.....
Family SICARIIDAE.

* Eyes usually 8, heterogeneous, either in three groups so composed that the median group consists of the two small diurnal direct eyes, while the lateral groups consist of the three indirect, nocturnal eyes each; or all eyes in a compact group. Direct eyes sometimes wanting. Chelicerae without boss. Margins transverse, with single tooth. Lip wide. Pedipalp in female without claw. Tarsi long, flexible, usually with several false segmentation lines. Upper claws similar, pectinate in a single row...Family PHOLCIDAE.

a. Carapace with head distinct, elevated. Thoracic groove present, longitudinal.....Sub-Family PHOLCINAE.

* Carapace uniformly convex. Head not distinctly separated. Thoracic groove wanting.....Sub-Family NINETIDINAE.

47. Eyes 6, nocturnal, in a compact group. Tarsi with onychium. Upper claws similar, pectinate in a single row. Lip immobile. Legs without spines. Chelicerae without boss. Margins toothed. Maxillary lobes converging. Posterior coxae separated by sternum..
Family LEPTONETIDAE.

a. Claw-tufts present. Pedipalp in female with claw. Maxillary lobes without serrula.....Sub-Family LEPTONETINAE.

* Claw-tufts wanting. Pedipalp in female without claw. Maxillary lobes with serrula.....Sub-Family OCHYROCERATINAE.

* Eyes 8, in rare instances six or wanting..... 48

48. External surface of chelicerae with stridulating organ. Thoracic groove longitudinal or wanting. Chelicerae without boss, with toothed oblique margins. Maxillary lobes about parallel. Claws similar, pectinate in a single row. Eyes heterogeneous..... Family LINYPHIIDAE.

a. Pedipalp in female with claw. Legs with spines..... Sub-Family LINYPHIINAE.

* Pedipalp in female without claw. Legs without spines..... Sub-Family ERIGONINAE.

* External surface of chelicerae without stridulating organ, with or without boss. Thoracic groove variable. Margins oblique, toothed. Maxillary lobes often wider at end than at base, rarely converging, usually parallel. Lip variable. Legs usually with stout spines. Claws similar or dissimilar, pectinate in a single row. Orbweavers.....Family ARGIOPIDAE.

a. Spinnerets surrounded by a chitinized wall. Abdomen always hard. Sub-Family GASTERACANTHINAE.[19]

* Spinnerets not surrounded by a chitinous wall..... *b*

b. Chelicerae without a boss, or boss rudimentary..... *c*

* Chelicerae with well developed boss..... *e*

c. Sternum broadly truncated behind. Legs without true spines. Femora without trichobothria. Eyes distinctly heterogeneous... Sub-Family THERIDIOSOMATINAE.[20]

* Sternum pointed behind. Legs with true spines..... *d*

d. Femora without trichobothria.....Sub-Family METINAE.

* Femora with several trichobothria..Sub-Family TETRAGNATHINAE.[21]

e. Tarsi with metatarsi longer than patellae with tibiae..... *f*

* Tarsi with metatarsi not longer than patellae with tibiae..... Sub-Family ARANEINAE.[22]

f. Lip longer than wide. Posterior row of eyes straight or recurved. Lateral eyes subequal.....Sub-Family NEPHILINAE.

*' Lip wider than long. Posterior row of eyes strongly procurved. Anterior laterals much smaller than posterior laterals..... Sub-Family ARGIOPINAE.

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[19] Includes Simon's GASTERACANTHEAE, MICRATHENEAE, XYLETHREAE and the genus *Tecmessa*.

[20] Includes also *Cyatholipus*.

[21] Includes TETRAGNATHEAE, PACHYGNATHEAE and LEUCAUGEAE.

[22] Includes Simon's ARGIOPINAE with the exception of ARGYOPEAE, GASTERACANTHEAE, MICRATHENEAE, XYLETHREAE, THERIDIOSOMATHEAE and the genera *Tecmessa* and *Cyatholipus*.

49. Chelicerae without a boss[23] 50
* Chelicerae with a distinct boss 51

50. Colulus wanting. Terminal joint of posterior spinnerets short. Anterior spinnerets usually considerably longer than posterior ones. Trochanteres slightly notched ..... Family ZODARIIDAE.
a. Anterior tarsi with scopula ..... *b*
* All tarsi without scopula ..... *c*
b. Spinnerets about equal. Anterior tarsi and metatarsi with scopula on inner edge only ..... Sub-Family HUTTONINAE.
* Anterior spinnerets much longer. All tarsi with usual scopula ..... Sub-Family STORENOMORPHINAE.
c. Posterior spinnerets longest. Tarsi with 2 claws ..... Sub-Family CITHAERONINAE.
* Anterior spinnerets longest. Tarsi with three claws ..... *d*
d. Claws smooth ..... Sub-Family CRYPTOTHELINAE.
* Claws with teeth ..... *e*
e. Eyes in two rows, both procurved, posterior median eyes being the third indirect eyes ..... Sub-Family ZODARIINAE.
* Eyes in three rows, posterior median eyes being the second indirect eyes ..... Sub-Family CYDRELINAE.

* Colulus present. Terminal joint of posterior spinnerets always long, sometimes quite unusually long. Posterior spinnerets always longer than anterior spinnerets. Trochanters not notched. Eyes on a tubercle, anterior laterals nocturnal, all others diurnal. Legs very long. Upper claws similar, pectinate in a single row ..... Family HERSILIIDAE.

51. Anterior pair of eyes small, approximated, situated far in advance of the other eyes near the edge of the clypeus. Anterior laterals alone nocturnal and much smaller than posterior laterals. Colulus present. Trochanters slightly notched. Integuments with plumose hair ..... Family SENOCULIDAE.
* Anterior pair of eyes not far removed from the other eyes 52

52. Tarsal trichobothria in a regular row. Legs without scopulae. Eyes 8, rarely 6 or 0, in two rows of four each, usually heterogeneous, direct eyes alone being of the diurnal type, sometimes almost homogeneous. Colulus present or wanting, terminal joint of posterior spinnerets coneshaped or cylindrical, often quite long. Trochanters usually not notched. Integuments with plumose hair. Upper claws pectinate in a single row ... Family AGELENIDAE.
a. Anterior spinnerets contiguous. Colulus present ..... *b*
* Anterior spinnerets not contiguous. Colulus wanting ..... *c*
b. Chelicerae without boss ..... Sub-Family NICODAMINAE.
* Chelicerae with boss ..... Sub-Family CYBAEINAE.
c. All spinnerets in a transverse row ..... Sub-Family HAHNIINAE.

[23] Except in *Nicodamus*.

* Arrangement of spinnerets normal.....Sub-Family AGELENINAE.

* Tarsal trichobothria either irregular or in two rows..... 53

53. Only the fourth trochanters with a slight notch. Eyes in two rows of which the anterior is strongly recurved and the posterior strongly procurved, together reminding of a hexagon. All eyes diurnal. Direct eyes always small. Clypeus high. Upper claws pectinate in a single row, inferior claw with 2 or 3 teeth. Integuments with scalelike hair.....Family OXYOPIDAE.

* All trochanters strongly notched. Posterior row of eyes always recurved 54

54. Upper claws with numerous teeth in a single row. Inferior claw with 2 or 3 teeth. Integuments with plumose hair. Legs longer, often laterigrade. Cocoon either carried by the female holding it with her chelicerae, or if attached to the spinnerets then hemispherical in shape.....Family PISAURIDAE.

* Upper claws with few teeth. Inferior claw smooth or with a single claw. Integuments usually with simple hair. Legs shorter, prograde without exception. Cocoon globular, carried by the female attached to her spinnerets. Posterior row of eyes usually so strongly recurved that the arrangement of the eyes may be said to be in three rows, in which case the four anterior eyes are much smaller.....Family LYCOSIDAE.

a. Eyes sub-equal in size. Posterior row very slightly recurved. Scopulae wanting.....Sub-Family RHOICININAE.

* The four anterior eyes much smaller. Posterior row so strongly recurved that it practically forms two rows of two eyes each.... *b*

b. Lower margin of chelicerae with four teeth. Eyes of third row elevated on tubercles. Scopulae wanting.....
Sub-Family BRADYSTICHINAE.

* Lower margin of chelicerae with two or three teeth. Eyes of third row not elevated on tubercles..... *c*

c. Anterior row of eyes strongly recurved. Scopulae wanting.....
Sub-Family CYCLOCTENINAE.

* Anterior row of eyes procurved or straight, rarely slightly recurved *d*

d. Sides of face vertical. Lip as wide as or wider than long. Anterior tarsi often with scopulae.....Sub-Family PARDOSINAE.

* Sides of face slanting. Lip longer than wide..... *e*

e. Terminal joint of posterior spinnerets very short, rounded. Integuments with simple hair. Anterior tarsi usually with scopulae...
Sub-Family LYCOSINAE.

* Terminal joint of posterior spinnerets longer. Integuments with plumose hair. Anterior and often all tarsi and metatarsi scopulate.....Sub-Family HIPPASINAE.

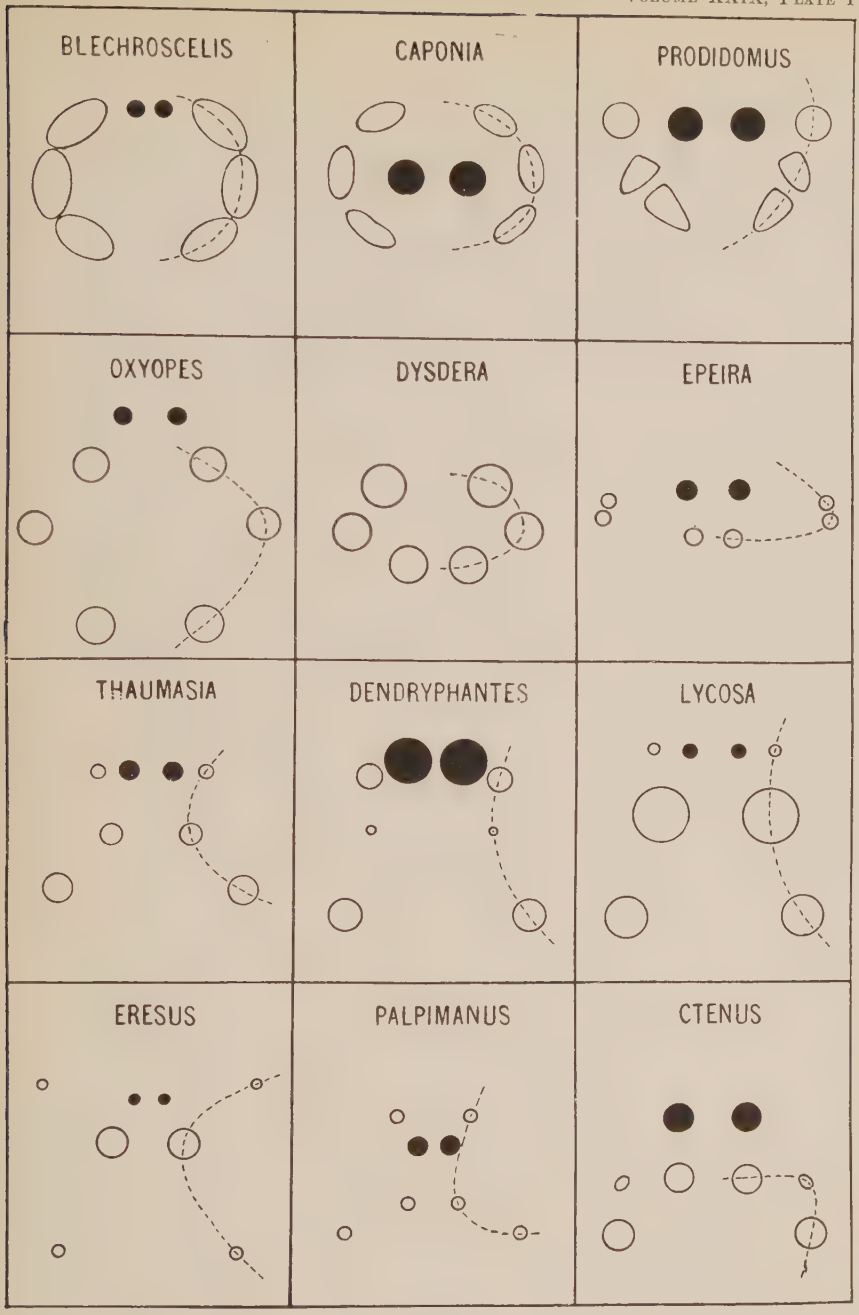

PLATE 1.—*Eyegroups in spiders, showing the three possible types of formation, dependent upon the relative position of the three indirect eyes of each side*

In the first type (*Blechroscelis, Caponia, Prodidomus, Oxyopes, Dysdera* and *Epeira*) the third indirect eyes furnish the pair of so-called posterior median eyes. In the second type (*Thaumasia, Dendryphantes, Lycosa, Eresus* and *Palpimanus*) the second pair of indirect eyes furnish the pair of posterior median eyes. In the third type (*Ctenus*) the first pair of indirect eyes furnish the pair of posterior median eyes. The dotted line connects the indirect eyes indicating their sequence as they receive their nerves from the corresponding neuromere. The direct eyes, usually called the anterior median eyes, are in all cases represented by black discs.

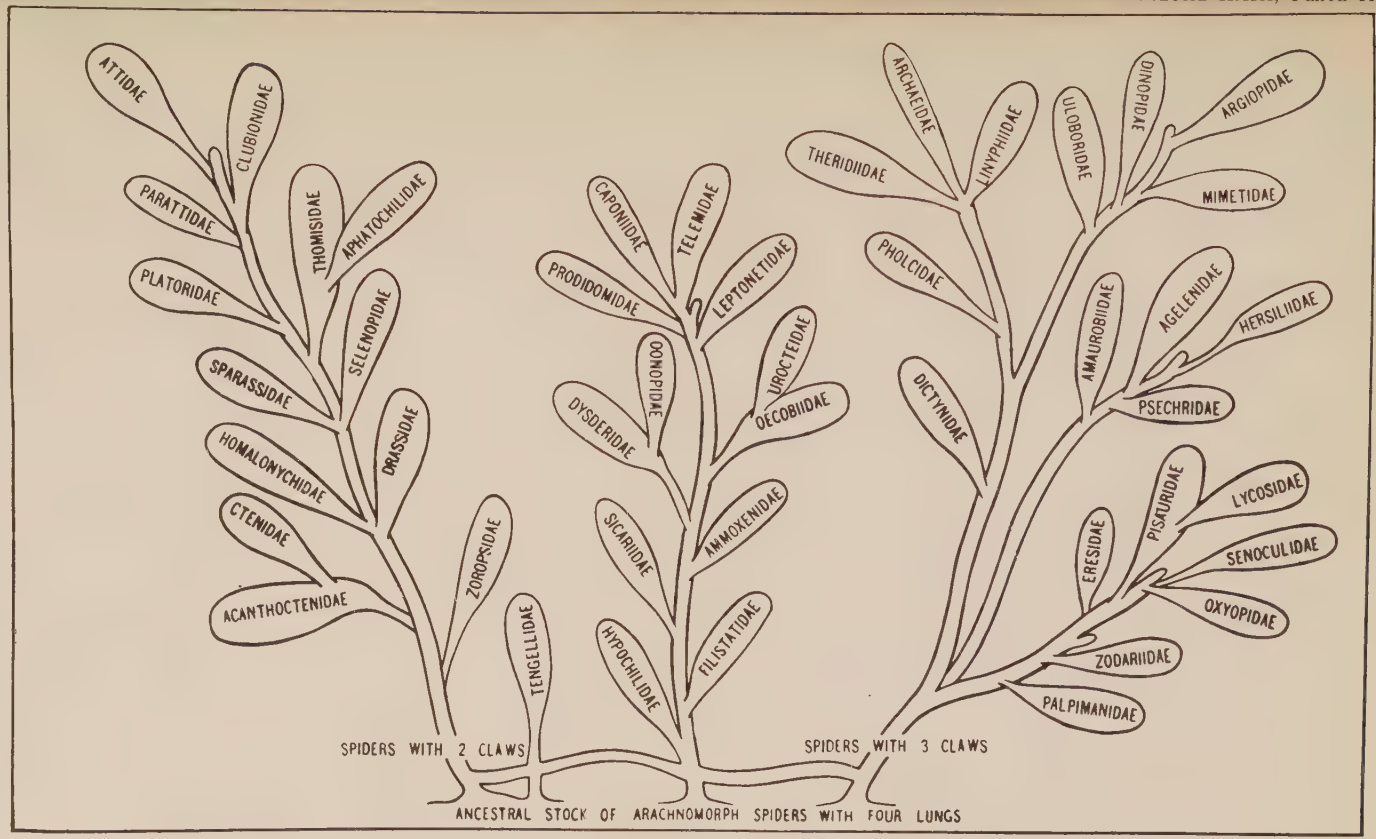

PLATE 2.—*Diagram showing the genetic relationship of spider families on the assumption that the ancestral stock consisted of arachnomorph spiders with two pairs of lungs, possessing a cribellum and calamistrum and three claws*

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The effect of thyroid products on Paramecium.

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Studies of Nowikoff, Shumway, and Budington and Harvey on the influence of thyroid products on Paramecium have apparently shown a marked increase in the rate of reproduction, and their results have been generally cited as a corroboration of the view that the active principle of the thyroid directly accelerates cell metabolism.[1] Recently Riddle and Torrey have failed to find an increased reproduction in Paramecium following subjection to thyroxin.[2]

We have reinvestigated the influence of thyroid products by a series of experiments on a pedigree race of Paramecium, using the organism as a "biological indicator" and following the general technique employed in other studies on Paramecium in this laboratory.[3]

The results, which will be published in detail elsewhere,[4] show

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1 Nowikoff, M., *Arch. f. Protistenkunde*, 1908, xi; Shumway, W., *Jour. Exper. Zool.*, 1914, xvii; *Ibid.*, 1917, xxii; Budington, R. A., and Harvey, H. F., *Biol. Bull.*, 1915, xxviii.

2 Riddle, M. C., and Torrey, H. B., *Proc. Amer. Soc. Zool., Anat. Rec.*, 1923, xxiv.

3 Woodruff, L. L., *Biochem. Bull.*, 1912, i; Woodruff, L. L., and Underhill, F. P., *Jour. Biol. Chem.*, 1913, xv.

4 *Journal of Biological Chemistry.*

in a clear cut manner that neither thyroxin (Squibb's) nor commercial dessicated thyroid, or fresh dessicated thyroid of the turtle produce any significant acceleration of the division rate of Paramecium.

Data to the contrary published by previous investigators apparently are attributable chiefly to variations in the bacterial food supply which the different media afforded the Paramecia.

Accordingly, all the evidence from studies on Paramecium to the effect that thyroid products accelerate cell anabolism is, we believe, erroneous.

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ON THE NATURE OF THE DETERMINING FACTORS IN REGENERATION

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THREE FIGURES

INTRODUCTION

This paper presents the results of an attempt to study separately certain dynamic factors concerned in regeneration, particularly as regards the regeneration of the head in pieces of Planaria dorotocephala. Incidental to contributions to the analysis of the principal problem, the data also include certain minor contributions to the knowledge of the effects of ether and chloretone on the rate of oxidative reactions in living protoplasm.

THE PROBLEM

Pieces of Planaria dorotocephala do not always regenerate anterior ends like that of the normal animal in nature. Abnormalities occur which are found to form a continuous series extending from the normal form to the headless form, which is devoid of anterior regeneration. For convenience in study this continuous series has been divided into five classes, Normal, Teratophthalmic, Teratomorphic, Anophthalmic, and Acephalic or Headless. This classification is based upon the degree of departure from the normal of the eyes, cephalic lobes, cephalic ganglia, and other structures of the regenerated anterior end; the forms have been described and figured elsewhere (Child, '11, '15; Child and McKie, '11). In the course of work involving the examination of literally hundreds of thousands of regenerated animals, no other types have been found. The numerical distribution of these types is fairly constant in mass experi-

ments consisting of pieces cut under as nearly as possible identical conditions with respect to length, region of section, and physiological condition of the animals, temperature, etc., differences between series being less than 10 per cent. This distribution is spoken of as the head frequency of the pieces under the conditions of the experiment. It varies greatly with the level from which the pieces are taken, with the length of the pieces, with the physiological condition of the animals, and with many other controllable conditions. Mass experiments therefore afford a convenient method of analysis of the factors concerned in determining the type of anterior end regenerated.

Much evidence indicates that in some way the quantitative physiological conditions in an isolated piece determine whether or not a head shall form. The present paper deals with the rôle in head determination of the rates of metabolic activity in various regions of the piece. It is necessary therefore to summarize briefly the more important facts that have been established concerning these conditions and also equally briefly what has seemed to the writer the most satisfactory interpretation of their relation to head determination. It has been found that when a piece is removed from the body of the animal certain changes in its rate of metabolic activity occur. These are:

First, the cells near the cut surfaces are stimulated by the act of section (Child, '14 a; Hyman, '19 c). This must be regarded in part due to wound stimulation and in part due to isolation from regions more anterior. Attention is confined in this paper to the conditions controlling the cells near the anterior cut surface, since they produce the new head. It is necessary to emphasize here that not only does wound stimulation affect these cells but also that they are isolated from the correlative and differentiative factors arising in more anterior regions. Isolation of parts or groups of cells in many organisms brings about rapid dedifferentiation and the initiation of development of a new organism. It is generally agreed that these processes involve increase in the rate of metabolic processes. The cells in question are not, however, completely isolated, for they are still anatomically and physiologically a part of a fragment of an

established system of correlations, and must be regarded as more or less under the correlative and differentiative control of that region of the piece which is not directly concerned in forming the new head and which is not directly affected by wound stimulation and isolation. For convenience in discussion, these cells near the anterior cut surface are designated as the X cells (fig. 1).

Second, it has been shown that the entire piece is stimulated by the act of section. This stimulation in regions not directly affected by wound stimulation is only temporary, passing away in less than forty-eight hours. It is marked by an increase in the rate of oxidation and is to be regarded as a transmitted

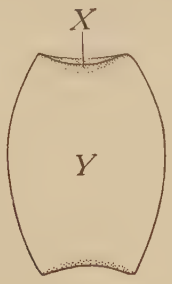

Fig. 1 An isolated piece of Planaria dorotocephala, showing *X*, the region near the anterior cut surface involved in wound stimulation, and *Y*, the remainder of the piece exclusive of the posterior cut surface.

stimulation, probably nervous in origin, and affecting the correlations of the original system that are still more or less operative in the isolated piece. It has also been shown that this stimulation is inverse to the length of the piece and that a piece from the posterior region of the first zooid is more stimulated than a piece of the same length taken from more anterior regions (Child, '14 a; Robbins and Child, '20; Hyman, '19 b, '22). For convenience the region of the piece in which the original system of correlations is still operative and which is involved in this transmitted, temporary stimulation is designated as the Y region (fig. 1).

It is obvious that if these two conditions exist they must be antagonistic, although the regions X and Y are anatomically and physiologically continuous; the X cells tend to dedifferentiate

and redifferentiate into the anterior end of a new individual, while the Y region carries on for a time and tends to maintain the correlations of the original individual.

The conception has been advanced by Child that the chief factor in determining whether or not a head shall form in an isolated piece of Planaria dorotocephala is the relation to each other of the rates of metabolism concerned in these two antagonistic physiological activities (Child, '14 b). In other words, if the rate of X in relation to that of Y is sufficiently high during the early period of dedifferentiation and redifferentiation, a normal head will form. On the other hand, if the rate of Y is sufficiently high in relation to that of X during this period, the developmental changes in the X region will be subjected to more or less inhibition and the resulting anterior end will fall in the series of abnormalities previously described (p. 395). If this is true, it should follow that an agent or condition which differentially affects the rate of metabolism of these two regions should play a part in head determination. By means of mass experiments, Child and others have shown that various physiological conditions of the animals do control head frequency according to expectations from this point of view. These conditions include age, mechanical stimulation, nutritive condition, and temperature. Quantitative measurements of the effect of several of these conditions upon the rate of physiological activity of the pieces appear to justify the conclusions relating the changes in head frequency produced to the relative rates of metabolic activity of the X and Y regions (Behre, '18; Child, '19 a, '19 b, '20 a; Hyman, '19 b, '19 c).

It has been stated above (p. 397) that the stimulation of the Y region by section may be regarded as temporarily augmenting the existing system of correlations in the piece. If this is true and if these correlative factors are really effective inhibitors of the development of the new head, it ought to be possible to increase head frequency by inhibiting this stimulation by means of depressing or anesthetic agents. Experimental evidence appears to justify this expectation, for Child was able to show that head frequency can be controlled by subjecting the pieces

to dilute solutions of KNC for a number of hours immediately after section (Child, '16), and it was shown by Hyman that such concentrations of KNC are powerful depressants of the rate of oxygen consumption of Planaria (Hyman, '19 a). After such treatment with KNC the head frequency of anterior pieces is decreased and that of posterior pieces of the first zooid is increased when compared with controls. Recently the writer extended this method of control to include a number of common anesthetics and showed that in concentrations of these agents that result in such changes in head frequency, the stimulation of the Y region by section does not occur, or is at least appreciably inhibited (Buchanan, '22).

On the basis of Child's conception, these results are interpreted as being due to the differential effect of the agent on the rates of metabolic activity of the X and Y regions. One effect may be called the direct effect, which inhibits the processes of dedifferentiation and development of the X cells by direct depression; this effect tends to reduce head frequency. Reductions appear in the head frequency of the anterior pieces because there the susceptibility of the X cells is probably greatest and because in anterior pieces the stimulation of the Y region is undoubtedly least and evidently has little effect on head formation, since head frequency is normally high in such pieces. The other effect may be called the indirect effect, which inhibits the stimulation of the Y region. The increases in head frequency which appear in posterior pieces of the first zooid are considered to result from this effect; in pieces from this level the stimulation of the Y region is greatest and the effect of the agent is to prevent or inhibit this stimulation and thus render less effective the correlative factors which in control pieces are apparently highly effective in inhibiting head formation, since in control pieces the head frequency of pieces from this level is very low. The stimulation of the Y region by section is therefore held to play an important rôle in determining whether or not a head shall form, since in its presence, as in posterior pieces of the first zooid, head frequency is low, and when it is slight, as in anterior pieces, or is inhibited as in depressants and anesthetics, head frequency is considerably higher.

The data on the effects of KNC on head frequency published by Child (Child, '16) and those published by the writer on the effects of chloretone, ether, and chloroform (Buchanan '22), indicate that there is a difference in the effects of these substances on the X and Y regions, and permit their division into two groups. KNC and chloretone, which may be regarded as general protoplasmic depressants, are very effective in producing decreases in head frequency as well as increases, while ether and chloroform, which are commonly regarded as anesthetics primarily affecting nervous elements, are much less effective in producing decreases in head frequency, but effective in producing increases. There is, however, no sharp distinction between the two groups; long-continued exposures to ether and to chloroform, especially the latter, bring about general protoplasmic depression and consequently decreases in head frequency. The effectiveness of ether and chloroform in producing increases in head frequency after exposures that are too brief to bring about general protoplasmic depression indicates that the stimulation of the Y region by section is largely of nervous origin since these substances affect nervous elements primarily.

It has seemed possible to subject this conception to still further analysis. If these are the chief factors concerned in head determination it should follow that subjecting the pieces after the stimulation of the Y region has disappeared to an agent that is effective in inhibiting nervous stimulation, but less effective in depressing the rate of metabolism of protoplasm in general, should have no effect on head frequency or at most only slight direct effects due to inhibition of cell division and growth of the young, highly susceptible tissues of the developing anterior end. It should also follow that the application of an agent that distinctly depresses the rate of metabolism in all regions of the piece should be effective in controlling head frequency even when applied after the stimulation of the Y region has disappeared. In other words, it should be possible by these means to study the relation to head determination of the metabolic rates of the X and Y regions in the absence of the temporary stimulation of the Y region.

METHODS AND DATA

To test these expectations experimentally it was necessary to find a stage in the regeneration of the pieces in which the stimulation of section of the Y region is no longer present, but in which regeneration has not proceeded to the extent that control of head frequency by experimental means is no longer possible. Child showed that in pieces regenerating in water under normal conditions head determination occurs during the first twelve hours after section (Child, '14 b) and during the period of stimulation of the Y region, a conclusion which received further support from the results of applying KNC and anesthetics during this period. Child and Hyman showed by several methods that the stimulation of the Y region disappears in less than forty-eight hours after cutting (Child, '14 a; Robbins and Child, '20; Hyman, '19 c, '22). No data were available as to whether or not head frequency could be controlled after this forty-eight-hour period, but for reasons that are obvious from the foregoing the period from the forty-eighth hour to the fifty-eighth hour was selected as the stage most likely to fulfill the necessary conditions.

The details of the manner of cutting the pieces have been described elsewhere and will not be redescribed here (Buchanan, '22). Figure 2 shows the regions from which the pieces were isolated. Animals of as nearly as possible the same size were selected from the same stock. The A, B, and C (fig. 2) pieces of fifty animals were placed in separate flasks, A in one, B in another, and C in another, and set aside for forty-eight hours. One such series served as a control. As time permitted, one or two other groups of fifty were cut in the same manner and placed on the same shelf with the control. At the end of forty-eight hours the water on the control pieces was changed and the water on the experimental series was replaced by the solution of the agent. After the desired period of time (four hours to nine hours) the solution was poured off, the pieces washed, and set aside to regenerate in water. In both controls and experimental series the water was changed every forty-eight hours and other condi-

tions were kept as nearly as possible identical. At the end of two weeks the number of each type of anterior end found in each series was recorded and will be referred to as the head frequency of the series. The head frequency of the series subjected to the agents can be compared only with that of the control cut on the same day and under the same conditions.

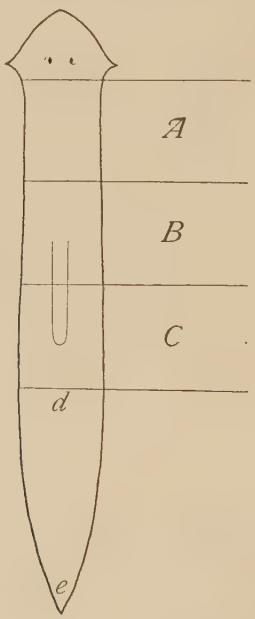

Fig. 2 Planaria dorotocephala, showing regions from which *A*, *B*, and *C* pieces were isolated. *d-e*, posterior zooids.

To meet the requirements of the conditions outlined for the problem it was necessary to find two agents, both known to inhibit the stimulation of the Y region and to control head frequency when applied during the period of stimulation, but one of which under the same conditions of period of exposure and concentration should be a general protoplasmic depressant when applied after the stimulation of section has disappeared, while the other should have no marked depressing effect. From data obtained in previous work it seemed probable that mol. 1/350 chloretone would fulfill the conditions for the first agent

and mol. 1/20 ether for the second. Little information on the effect of chloretone on oxygen consumption is available, but it was shown by the writer that when pieces are subjected to mol. 1/350 chloretone for a number of hours immediately after section not only is the increase of oxidations attendant upon section inhibited, but the rate of oxygen consumption of the pieces is lower than that of intact animals. On the other hand, while mol. 1/20 ether was shown to inhibit the stimulation of section, there was little indication that the rate of oxygen consumption of the pieces was reduced within the time limits below that of the normal intact animals (Buchanan, '22).

To test the effect of these two agents on the rate of oxygen consumption of the pieces after the forty-eighth hour following section, two series of experiments were conducted in which the pieces were cut as in the head-frequency experiments, but instead of the pieces from different levels being separated, they were placed in the same flask. After standing in water for forty-eight hours their total oxygen consumption in water over a period of hours was measured. The water was then replaced by mol. 1/350 chloretone in one series of experiments and by mol. 1/20 ether in the other, and the oxygen consumed in these agents was measured over a certain period, four, five, or six hours. The amount of oxygen in solution in the water and in the anesthetic solutions was measured by the Winkler method. The exact procedure is described elsewhere (Hyman, '19 a; Buchanan, '22).

The results of these two series of experiments are given in table 1.

It will be seen that mol. 1/350 chloretone does actually decrease the total oxygen consumption quite distinctly in all cases, from which it is reasonable to conclude that the rate of oxidative reactions is depressed. Mol. 1/20 ether apparently has little effect; slight increases occur which may be due to increased motor activity. The fact is appreciated that these data show only the total oxygen consumption and in no way measure the possible fluctuations in rate during the period of exposure. The criticism may arise that these are inadequate grounds for assert-

ing that no depression of rate occurs in mol. 1/20 ether. There is evidence to show that deep anesthesia may be produced by similar concentrations of ether without appreciably decreasing the rate of oxygen consumption (Loeb and Wasteneys, '13 b). In mol. 1/20 ether the anesthesia produced in pieces of Planaria is not profound; ciliary movements are appreciably inhibited and the pieces remain helpless at the bottom of the flask, but irregular muscular movements continue. Motor activity of this sort introduces a complicating factor in the measurement of oxygen consumption in ether solutions. It was found in previous work and preliminary experiments that motor activity immediately after section in pieces that are approximately one-third the first zooid is not sufficient to obscure the inhibition by solutions of ether of the increase of oxygen consumption attendant upon section. With longer pieces in which the stimulation is less and the motor activity greater, this effect is masked by the oxygen consumption due to the writhing of the pieces. Measurement of the oxygen consumption of whole animals by this method is also unsatisfactory for the same reason. In short pieces subjected to mol. 1/20 ether forty-eight hours after section measuring the oxygen consumed by hourly tests is unsatisfactory apparently because the pieces have more or less recovered from the shock of cutting and the introduction of the liquid at the start of each hour period as is necessary by this method violently agitates the pieces. Control pieces in water are not so agitated and tossed about in the flask, for they adhere firmly to the wall of the flask. The results of hourly tests in ether are irregular, ranging from slight increases in oxygen consumption to 100 per cent increases. It may be stated here that in no case either in this work or in previous experiments have I found that mol. 1/20 ether appreciably depressed the rate of oxygen consumption below that of the control within limits of duration of exposure that are employed in the experiments reported in this paper. It is therefore regarded reasonable to conclude that the data given in table 1 indicate that marked depression of rate of oxidation reactions does not occur in mol. 1/20 ether within the time limits stated. On the strength of these data, these two solutions, mol.

1/350 chloretone and mol. 1/20 ether, were used in the head-frequency experiments and applied as previously described (p. 401).

The effects on head frequency are shown in table 2 and table 3. A comparison of these results with the effect of the agents on the oxygen consumption given in table 1 shows plainly that in mol. 1/350 chloretone both increases and decreases in head frequency are associated with depression and that mol. 1/20

TABLE 1

Total oxygen consumption, pieces. Forty-eight hours after cutting

EXPERIMENT	AGENT	WEIGHT	TEMPERATURE	TIME	TOTAL OXYGEN CONSUMPTION IN WATER	TOTAL OXYGEN CONSUMPTION IN AGENT
		mgm.	*degrees*	*hours*	*cc.*	*cc.*
1	M/350 chloretone	293	21	4	0.334	0.210
2	M/350 chloretone	275	21	4	0.313	0.214
3	M/350 chloretone	280	21	4	0.324	0.145
4	M/350 chloretone	252	18	5	0.333	0.264
5	M/350 chloretone	272	18	5	0.340	0.292
6	M/350 chloretone	285	18	5	0.356	0.185
7	M/20 ether	319	17	4	0.274	0.287
8	M/20 ether	257	17	4	0.195	0.236
9	M/20 ether	340	17	4	0.285	0.350
10	M/20 ether	376	22	6	0.597	0.631
11	M/20 ether	410	22	6	0.713	0.713
12	M/20 ether	402	22	6	0.651	0.735

Length of whole animals 16 to 18 mm. except in nos. 4, 5, and 6, in which length was 14 to 16 mm.

Fifty animals used in each experiment.

Animals fed forty-eight hours before cutting.

ether, which is not effective in lowering the oxygen consumption, is also not effective in increasing head frequency; only decreases appear.

It is possible to show these effects on head frequency by means of a graph (fig. 3). Plotting the data in no. 41 and no. 42, table 2, for example, we may assign the arbitrary value of 5 to each normal form, 4 to each Teratophthalmic, 3 to each Teratomorphic, 2 to each Anophthalmic, and 1 to each headless. On this basis

TABLE 2

Head frequency, pieces treated with chloretone after forty-eight hours after section, in per cent

SERIES	TIME	PART	NORM.	T. OPTHTH.	T. MORPH.	AN.	HDL.	DEAD	RESULT
August 16, 1921. 16–18 mm. worms. Fed four days before cutting									
	hours								
No. 11, control		A	78	22					
		B	2	86		12			
		C	6	52		42			
No. 12, M/350 chloretone	4	A	88	12					+
		B	6	72	2	20			0
		C	6	60		34			0
No. 13, M/350 chloretone	4	A	84	16					0
		B	20	54	4	22			+
		C	6	58		36			0
August 11, 1921. 12–14 mm. worms. Fed three weeks before cutting									
No. 21, control		A	80	20					
		B		34	2	64			
		C		20		80			
No. 22, M/350 chloretone	$5\frac{1}{2}$	A	94	6					+
		B	6	70	2	22			+
		C	8	40		52			+
No. 23, M/350 chloretone	$5\frac{1}{2}$	A	96	4					+
		B	12	48	2	38			+
		C	2	28		70			+
December 18, 1920. 16–18 mm. worms. Fed three days before cutting									
No. 31, control		A	76	22				2	
		B		54	4	36		6	
		C	2	36		32	28	2	
No. 32, M/350 chloretone	$5\frac{1}{2}$	A	72	20				8	0
		B	18	62	4	10		6	+
		C	18	38	4	34	4	2	+

TABLE 2—*Continued*

SERIES	TIME	PART	NORM.	T. OPTRTH.	T. MORPH.	AN.	HDL.	DEAD	RESULT
August 2, 1921. 18–20 mm. worms. Fed four days before cutting									
	hours								
No. 41, control		A	86	14					
		B	6	58	2	34			
		C	2	38		52	8		
No. 42, M/350 chloretone	5½	A	96	2			2		+
		B	16	62		22			+
		C	4	62	2	32			+
No. 43, M/350 chloretone	5½	A	96	4					+
		B	16	72		12			+
		C	10	62	4	22	2		+
August 16, 1921. 14–16 mm. worms. Fed four days before cutting									
No. 51, control		A	62	38					
		B		62		38			
		C		34		56	10		
No. 52, M/350 chloretone	5½	A	80	20					+
		B	10	44	4	42			+
		C	8	40	2	40	10		+
No. 53, M/350 chloretone	5½	A	80	20					+
		B	4	66		30			0
		C		32		68			0
August 2, 1921. 18–20 mm. worms. Fed four days before cutting									
No. 61, control		A	90	10					
		B	4	78		18			
		C	2	50		28	12	8	
No. 62, M/350 chloretone	8	A	84	16					0
		B	18	58		24			−
		C	2	34		60	2		−
No. 63, M/350 chloretone	8	A	90	10					0
		B	12	52		36			−
		C	6	36		46	12		−

TABLE 3

Head frequency, pieces treated with ether after forty-eight hours after section, in per cent

SERIES	TIME	PART	NORM.	T. OPTHTH.	T. MORPH.	AN.	HDL.	DEAD	RESULT
May 28, 1921. 16–18 mm. worms. Fed one week before cutting									
	hours								
No. 71, control		A	82	18					
		B	4	88		8			
		C	18	70		12			
No. 72, M/20 ether	6	A	68	32					−
		B	10	76		12		2	0
		C	18	70	2	10			0
No. 73, M/20 ether	6	A	60	40					−
		B	6	72	2	20			−
		C	18	50	2	30			−
June 27, 1921. 16–18 mm. worms. Fed two weeks before cutting									
No. 81, control		A	100						
		B	6	64	2	28			
		C	6	38		52	4		
No. 82, M/20 ether	6	A	96	4					0
		B	10	66	2	22			0
		C	8	58	2	28	4		+
No. 83, M/20 ether	6	A	98	2					0
		B	10	62		28			0
		C	6	32	2	50	10		0
June 15, 1921. 14–16 mm. worms. Fed ten days before cutting									
No. 91, control		A	80	16				4	
		B	40	46		10		4	
		C	8	46		42		4	
No. 92, M/20 ether	8	A	82	12				6	0
		B	42	54				4	+*
		C	2	44		50		4	0
No. 93, M/20 ether	8	A	80	12				8	0
		B	40	54		4		2	0
		C	8	56		32		4	+

* Slight.

TABLE 3—*Continued*

SERIES	TIME	PART	NORM.	T. OPTHTH.	T. MORPH.	AN.	HDL.	DEAD	RESULT
June 4, 1921. 15–17 mm. worms. Fed one week before cutting									
	hours								
No. 101, control		A	92	4				4	
		B	2	76		22			
		C		28	4	64	4		
No. 102, M/20 ether	9	A	80	20					—
		B	2	70		24		4	0
		C		36	4	60			0
No. 103, M/20 ether	9	A	70	30					—
		B	2	76	4	18			0
		C		20		76	4		0

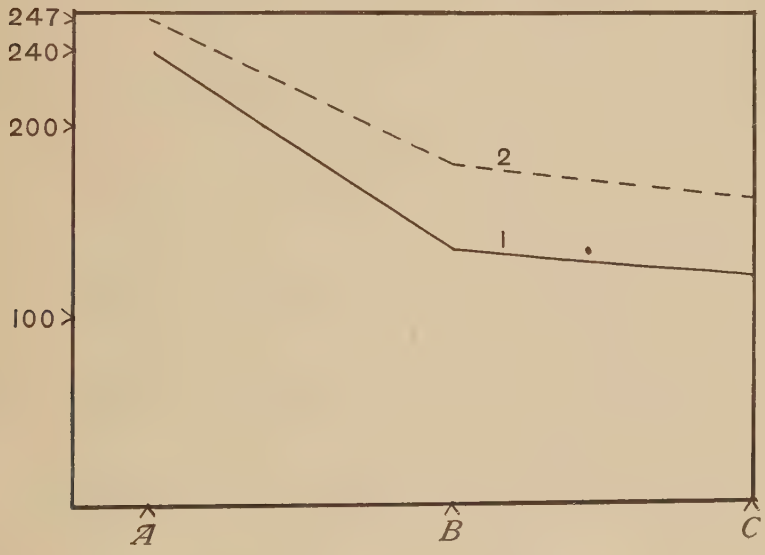

Fig. 3 Curves showing head frequency of a series of pieces after exposure to mol. 1/350 chloretone, no. 2, and its control, no. 1. *A*, *B*, *C*, anterior cut surfaces of *A*, *P*, and *C* pieces, respectively. The ordinates are the values of head frequency. For method of calculation of ordinates, see text.

the value of the A pieces in the control is 240, forty normal animals and ten teratophthalmic, B pieces have a value of 135, C pieces the value of 120, which are plotted as ordinates, the approximate distance between the anterior cut surfaces being plotted as the abscissae. On the same basis of valuation the value of A pieces after treatment with chloretone is 247, of B pieces 180, and of C pieces 152. No claim is made that the values 5, 4, 3, 2, 1, represent any exact mathematical valuation of regenerated tissue; this is simply a convenient and consistent way of showing the effects of the agent on head frequency.

Chloretone. Closer inspection of the data on the effects of chloretone on head frequency reveals their consistent nature. Disregarding differences between the control and experimental series of less than 10 per cent as falling within the limits of error, it is noted that short periods of exposure, four hours, no. 12 and no. 13, show no effect when compared with their control except in two cases, a slight increase in the head frequency of the A pieces in no. 12 and a slight increase in B pieces in no. 13. The expectation is that increases should appear first in pieces in which the Y region has the least effect on head formation, i.e., in pieces from anterior regions. Five-and-one-half-hour exposures are evidently effective in bringing about sufficient indirect effect in pieces from all three regions, since in nos. 22, 23, 32, 42, 43, 52, 53, increases in head frequency occur in A, B, and C pieces except in B and C, no. 53, in which there is little effect.

Eight-hour exposures, no. 62, no. 63, bring about direct effects. Evidently the X region of A pieces is better able to recover from the direct effect of the agent, since decreases are least in these pieces. This indicates that the metabolic rate of the X region of anterior pieces[1] is higher than that of the corresponding region of more posterior pieces, a probability which Child has already noted (Child, '16). Analysis of the head frequency of C pieces in no. 62 and of B and C pieces in No. 63 reveals that in these

[1] Extended investigations by Child, Hyman, Bellamy, and others have shown that regions of high rate of metabolism acclimate to or recover from the effects of relatively strong solutions more readily than do regions of lower rate. For references and a résumé of the evidence, see Child, '20 b.

cases both direct and indirect effects are shown. The head frequency of some of the pieces is shifted upward, while in the majority it is shifted downward.

Ether. In considering the data on the effects of ether on head frequency it is necessary only to point out that this agent which was shown to be highly effective in increasing head frequency when applied during the period of stimulation has very little effect when applied after the stimulation has disappeared. Increases of 10 per cent or more occur in only three out of possible twenty-two cases, no. 92 B, no. 93 C, and no. 95 C. Decreases occur in six cases out of twenty-four. One is justified in stating that for some reason mol. 1/20 ether is not effective in increasing head frequency, although effective in producing decreases.

DISCUSSION

Dealing first with the effects of mol. 1/20 ether, we have to consider, 1) that when it is applied during the early period of stimulation of section the head frequency of A pieces is slightly decreased, while that of C pieces is distinctly increased (Buchanan, '22); 2) that when applied after the stimulation of section has disappeared, more decreases appear in A pieces and practically no increases appear in C pieces; 3) mol. 1/20 ether inhibits the increase in rate of metabolism that normally follows section; 4) mol. 1/20 ether is not effective in producing general protoplasmic depression within the time limits employed in these experiments. A consideration of these experimental data, in the light of the fact that ether is primarily a narcotic of nervous elements, leads unavoidably to the conclusions, 1) that the stimulation of the Y region by section is an important factor in determining whether or not a head shall form at X; 2) that the stimulation of the Y region by section is largely of nervous origin; 3) that ether is not effective in producing increases in head frequency when applied after the stimulation of section has disappeared, because it has little depressing effect on protoplasm in general, and hence is not effective at this time in decreasing the effectiveness of the correlative factors arising in the Y region

that tend to inhibit the processes of differentiation and development going on in the X cells and their progeny.

As regards the decreases in head frequency that occur in A pieces, the case seems clear that these result from direct inhibition of the processes of development of the X cells. That inhibition of cell division may be brought about by solutions of anesthetics which do not appreciably affect the rate of oxygen consumption was shown by Loeb and Wasteneys in the arrest of cleavage of Arbacia eggs by various anesthetics (Loeb and Wasteneys, '13 a), also by Warburg in the effect of certain concentrations of phenyl urethane on cleavage of Stronglyocentrotus (Warburg, '10). It is possible that in decreases in head frequency produced by a solution of ether that does not appreciably inhibit oxidations in general we have evidence of a similar phenomenon. What appears more probable is that these decreases are due to inhibition of the metabolic rate of the young, highly susceptible tissues that is not detectable in the measurement of the oxygen consumed by the whole piece. Explanation why this effect is greater after forty-eight hours is to be found in the fact that the X cells are more susceptible to many external agents, including ether, at this time than immediately after section. The processes of growth and cell division which are going on in this region after forty-eight hours involve increased metabolic activity and hence increased susceptibility (footnote, p. 410).

The data on head frequency show that some sort of difference exists between the effect of ether and that of chloretone when applied after forty-eight hours. The data on the effects of these agents on oxygen consumption show that at least one difference in their effects on the physiological conditions of the pieces at this time is that chloretone materially reduces the rate of oxidations of the whole of the piece. A brief consideration of the evidence will show that this difference in effect on oxidative reactions is sufficient to account for the difference in effect on head frequency. Inhibition of the stimulation of the Y region during the period following section by KNC, alcohol, chloroform, chloral hydrate, chloretone, ether, temperature, and other

conditions has been shown to result in increases in head frequency in pieces from regions in which this stimulation is normally relatively great. One is justified in regarding the Y region as the locus of a system of correlations inherited from the animal from which the piece is taken. A priori there are no reasons for believing that this system of correlations has disappeared after forty-eight hours, although its effectiveness probably decreases during this time. The two antagonistic physiological activities, the tendency of the X cells to develop into the anterior end of a new individual and the tendency of the Y region to maintain the correlations of the old individual, are still existent in the pieces. Chloretone, being a general protoplasmic depressant, depresses the metabolic activity of both the X and Y regions, but the degree of depression and rapidity of recovery are not equal in each. The X cells, being in the process of growth, cell division, and differentiation, are much more active metabolically than the cells of the Y region and are therefore much more susceptible to the depressing agent, but *recover much more quickly* (foot-note, p. 410). Recovery of the X cells and their resumption of the processes of growth and differentiation while the Y region is still in a state of depression are conditions that make for independence of X cells from the inhibitory influence of the correlative factors arising in the Y region, and consequently account for increases in head frequency.

As regards the decreases in head frequency which appear in the series treated with chloretone, it may be pointed out that these are greater the longer the exposure to the chloretone. This suggests that the X cells are not only depressed more than the Y region by such treatment, but also that these young, highly susceptible tissues undergo such a profound depression, and perhaps injury as well, that their recovery is less complete than that of the less susceptible Y region. The decreases in head frequency may be considered to result from this extreme depression of the X cells from which recovery is incomplete.

These results are incompatible with explanations of regeneration based on the distribution or presence of nutritive materials or of specific formative substances. Since it is possible to

increase head frequency experimentally by the application of appropriate concentrations of chloretone for a certain period forty-eight hours after section, we cannot assign the absence of increases after treatment with ether at this time to the disappearance of a head-forming or nutritive substance. The difference in the effectiveness of ether as an agent in increasing head frequency when applied immediately after section and when applied forty-eight hours later must be due to the disappearance of a condition, not to the disappearance of a substance. Nor can we assign the effectiveness of chloretone as an agent in producing increases in head frequency to its specific reaction with a specific substance, since a wide range of substances and of physiological conditions have been shown to be equally effective in producing increases in head frequency when applied immediately after section.

These data, when considered in the light of the body of facts that has accumulated, constitute evidence that strongly supports Child's conclusion, that head determination in Planaria dorotocephala is dependent on the relation to each other of the rates of metabolic activity of the X and Y regions. These data also show that, while head determination occurs normally in water during the first few hours after section, the fate of the X cells is not absolutely fixed but may be modified at a later period by a depressing agent. It would be interesting to compare the control of head frequency by this means as regeneration proceeds with the morphological changes associated with the reorganization of the old tissues of the piece into their definitive parts in the reconstituted individual.

SUMMARY

Concentrations of chloretone that have been shown to be effective in controlling head frequency in pieces of Planaria dorotocephala when applied immediately after section during the period of stimulation are also effective in controlling head frequency when applied after the stimulation of section has disappeared.

Concentrations of ether that are effective in controlling head frequency when applied immediately after section during the period of stimulation are *ineffective* in producing increases in head frequency when applied after the stimulation of section has disappeared; only decreases in head frequency appear.

Experimental evidence is presented to show: 1) That chloretone in the concentration and duration of exposure employed in the head-frequency experiments is an effective general protoplasmic depressant; 2) that ether in the concentration and duration of exposure employed is not effective in producing general protoplasmic depression.

The facts indicate, 1) that the young, metabolically more active tissues of the developing anterior end recover more rapidly from the depressing effect of moderate exposure to chloretone than do the less active older tissues of the piece; this recovery and resumption of the processes of growth and differentiation while the remainder of the piece is still in a state of depression tends to increase head frequency; 2) that long-continued exposure to the depressing action of chloretone so affects the young, highly susceptible tissues of the developing anterior end that recovery is incomplete; this effect tends to decrease head frequency; 3) that no increases in head frequency appear after exposure to ether because this substance is not effective in depressing the metabolic activity of the old tissues of the piece; apparently only the highly susceptible tissues of the developing anterior end are appreciably affected; hence only decreases in head frequency appear.

The data also indicate that the stimulation of the piece, exclusive of the cells near the cut surfaces, immediately following section, demonstrated by Child and also by Hyman by a number of methods, is largely of nervous origin.

The evidence strongly supports the conclusion reached by Child that head determination in pieces of Planaria dorotocephala depends on the relation of the metabolic rate of the cells near the anterior cut surface of the piece from which the new anterior end is derived to that of the remainder of the piece.

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INTERSEXES IN NEMATODES

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STIMULATED by the experiments of Goldschmidt on the Gypsy Moth the writer carried out extensive experiments in the winter and spring of 1921-22, in which he attempted to cross several morphologically closely related free-living nematode species.[1] The chief aim was to see if intersexes (as the term is used by Goldschmidt) could be produced by hybridization of certain easily reared species.

Intersexes are individuals which possess the sexual characters of one sex mixed with the characters of the other. Such cases have been long known and some of them were designated hermaphrodites, gynandromorphes, etc. Modern experiments have showed that whole series occur in which the characters of a male and female are more or less mixed, thus representing all possible steps between a true female and a true male.

Mention of some intersexes already existed in nematode literature; there were several short accounts of them in some species of *Mermithidae*, and a description of those reported in other genera is given in later paragraphs of this paper.

Unfortunately my experiments did not have the desired results. It seems to be very difficult to cross the species of the genera mentioned in the foot note. Thus far my experience agrees with that of Maupas, published in a posthumus paper edited by Caullery.[2]

During the last few months the writer was called upon to identify a large number of Mermithidae. Numerous intersexes were observed. Since the sex problem is today a center of biological discussion, a description of these intersexes is certainly of some interest at this time.

Intersexes in the Hair-Worm

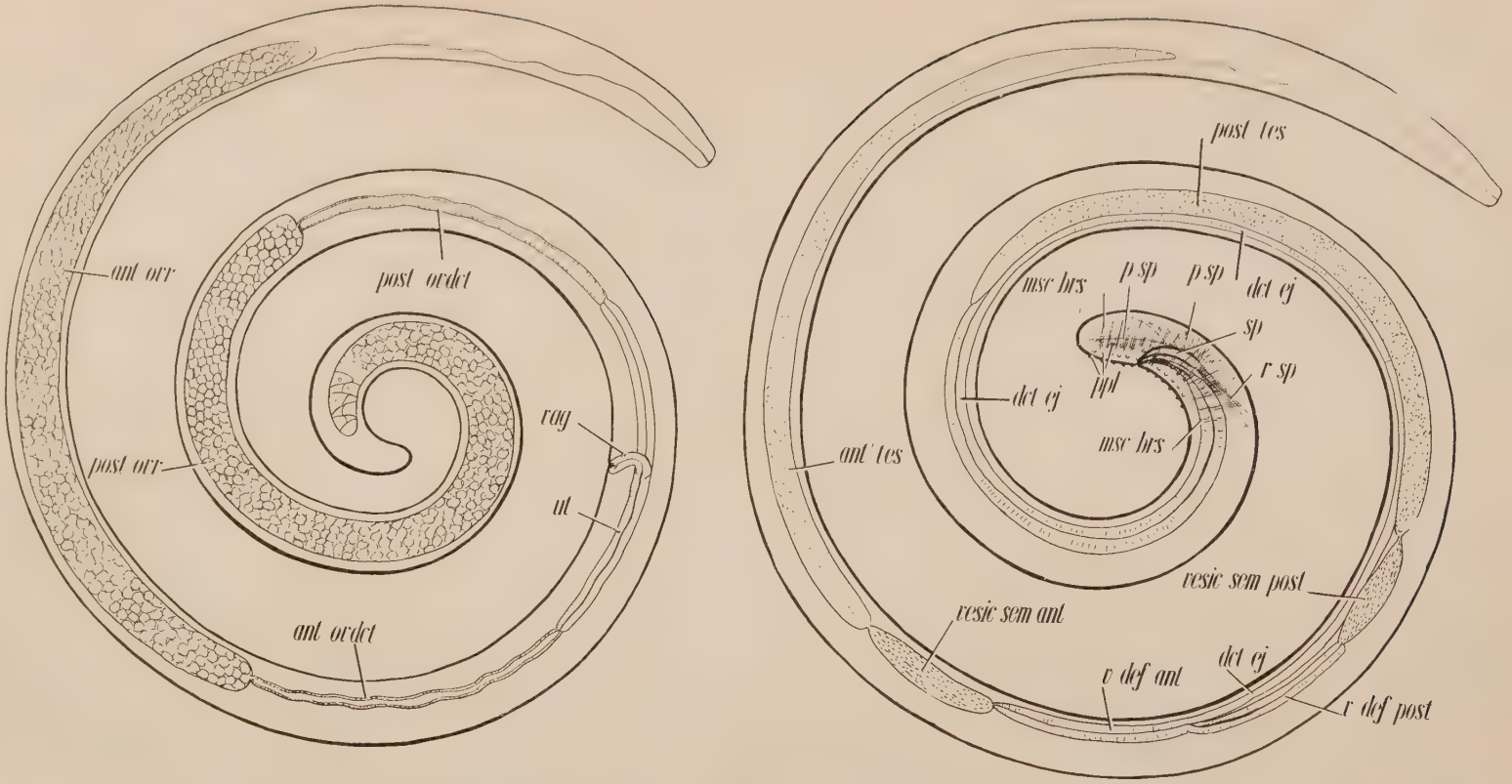
Hitherto every investigator who has studied any considerable number of *Ag. albicans*[3] or related forms, mentions the presence of intersexes[4] in this species. Meissner in his splendid study of this form describes as early

[1] For the opportunity to carry on these experiments I am greatly indebted to the authorities of Yale University and the staff of the Osborn Zoological Laboratory, New Haven, Conn. Species of Rhabditis, Plectus, Cephalobus and Diplogaster were used in these experiments, an account of which will be given in a later paper.

[2] Maupas, Emile, Essay d'Hybridation chez des Nematodes. Bull. Biol. de la France et de la Belgique. T. 52. 1919.

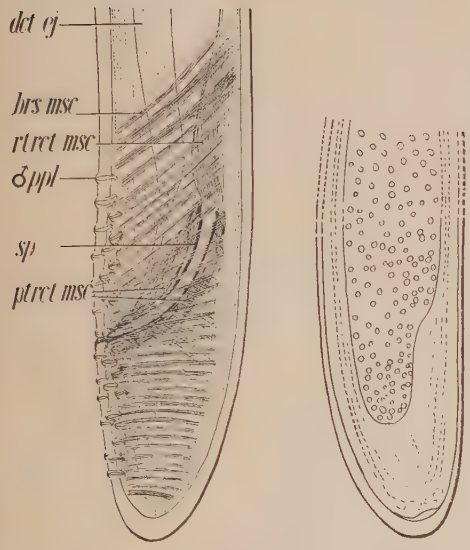
[3] During recent experiments it was found best to split the former genus Mermis into several new genera. In the future the genus Mermis will include only those forms with four head papillae, two mouth papillae, amphids situated laterally in the same level with the head papillae, mouth terminal, vagina S-shaped; type species *M. nigrescens Duj.* The new genus Agamermis will include forms with six head papillae, no mouth papillae, amphids mostly behind the lateral head papillae, or slightly shifted dorsad, mouth terminal, vagina S-shaped. In entering the host the preparasitic larva of this genus drops five-sixths of its body through self-amputation at a preformed node. Our type species, *Ag. decaudata* resembles very much the former *Mermis albicans* as described by Meissner and partly by Hagmeier, but differs from it through the structure and position of the amphids or lateral organs, and the cells around the esophagus. Furthermore it is not known whether in the latter form the tailpart of the larval body is dropped by self-amputation, as in *Ag. decaudata.* We are sure that *M. albicans* of earlier investigators includes perhaps a number of different species. For further details, see the preliminary paper: Cobb, Steiner, and Christie, *Agamermis decaudata,* a Nema Parasite of Grasshoppers and Other Insects. *Journal of Agricultural Research,* Vol. 23, No. 11, 1923.

[4] They were not termed such, but were called hermaphrodites or "Zwitter."



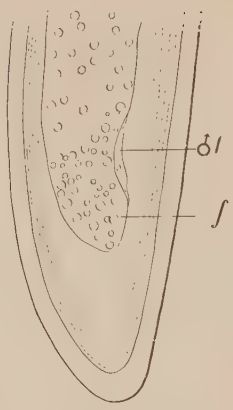
INTERNAL STRUCTURE OF MALE AND FEMALE HAIRWORMS

FIGURE 1. These outline drawings are not strictly to scale and only the reproductive organs are shown, these being the parts of most interest in connection with this article. The nematodes are such slender animals that paired organs would have no space to lie side by side, and so are arranged "tandem." The nematodes offer a unique field for the experimental study of many biological problems. Under the microscope the physiologist can actually observe many metabolic processes that he can study only indirectly in larger organisms. More variations in reproductive processes and in the sex ratio are found in nematodes than in any other animals. Species are known in which no males or only one male is produced to five hundred females, and others in which ten times more males occur than females, and between these extremes are species in which any intermediate ratio can be found. *ant. ovr,* anterior ovary; *ant ovdct,* anterior oviduct; *post ovr,* posterior ovary; *post ovdct,* posterior oviduct; *ut,* uterus; *vag,* vagina; *ant test,* anterior testis; *dct ej,* ductus ejaculatorius; *msc brs,* bursal muscles; *ppl,* male copulatory papillae; *p sp,* protractor muscles of the spicula; *r sp,* retractor muscles of the spicula; *sp,* spiculum; *v def ant,* anterior vas deferens; *v def post,* posterior vas deferens; *vesic sem ant,* anterior seminal vesicle; *vesic sem post,* posterior seminal vesicle.



TAIL END OF MALE AND FEMALE ~~NEMATODES~~ Ag. decaudata

FIGURE 2. Intersexes are individuals having the reproductive organs of one sex and, in addition, certain organs or tissues typical of the other. All of the intersexes so far observed in nematodes have been females in which certain typically male parts are present. Above is shown the tail end of a normal female (right) and of a normal male (left), showing the male organs that are found in intersexual females (Figures 5-8). *dct ej,* ejaculatory duct; *brs musc,* bursal muscle; *♂ ppl,* male papilla; *ptrct msc.,* protractor muscle; *rtrct msc,* retractor muscle; *sp, spiculum.*



A SLIGHTLY INTERSEXUAL FORM

FIGURE 3. Tail end of female *Ag. decaudata,* showing a small amount of male tissue (♂ t). Goldschmidt experimentally produced "consecutive intersexuality" in moths by crossing related races. These individuals would begin life as females and gradually change to males, at the same time losing their female characters. He accounted for this change by assuming that the sex determiners of the different races used in the experiment had a different relative rate of activity, and that an initially slow-working "male factor" would gradually increase in activity until it overshadowed the "female factor," when the individual would change from a female to a male.

as 1854 three intersexual females and is very much puzzled over this unusual combination of male and female characters.

The majority of the specimens of *Agamermis decaudata* which I have had the opportunity to study came from Falls Church, Virginia, and were collected by Mr. J. R. Christie. It was very striking that the specimens with intersexual characters were as numerous as, or even more numerous than, normal females.

One important fact, to be noted at the outset, is that *hitherto only intersexual females have been observed,*— that is, all specimens with intersexual characters were females; the female sex organs were completely and normally developed. Vulva, vagina, uteri and ovaries were normal and in most of the animals eggs were present in the oviducts and uteri. Up to the present the writer has never seen a male with intersexual characters, and, as far as he knows, no other investigator has.

Let us now first see what a normal female and male of this species look like:

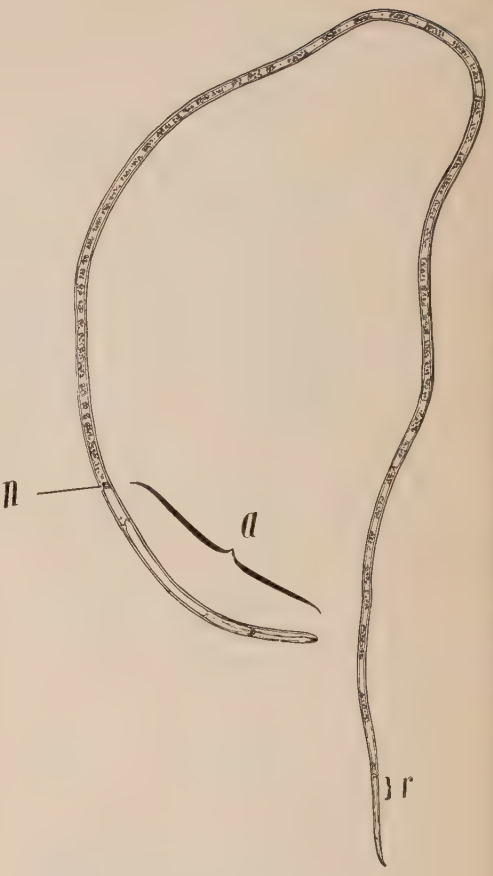
The two sexes may be distinguished from each other with the naked eye, as they are in most cases distinctly different in size. The photographs, (Frontispiece), show a female and several males that were found together in the soil. The female is several times larger than the male. The average length of six females was found to be 293.7 mm., ranging from 190 to 380 mm., whereas the average length of ten males was only 63.5 mm., rang-

ing from 42 to 104 mm. Hagmeier gives for the males of *Ag. albicans* a length from 29 to 80 mm., for the females 38 to 320 mm. Occasionally very small females may also be seen in this species exceeding in length only slightly, or not at all, the average male. Unusually large males have also been observed, attaining the length of the smaller females. While the size may therefore have a certain value in determining the sex of individuals the gonads and copulatory organs are nevertheless the chief characters. The female sexual opening, the vulva, is a narrow transverse fissure near the middle of the body. It leads into a tubular vagina opening at right angles into the uteri. They are outstretched, one forward the other backward, and merging through the oviducts into the ovaries (Fig. 1).

The male sex opening, however, is situated near the tail end, and combined with a copulatory apparatus consisting of two cutinized spicula and the muscles needed to move them. The *ductus ejaculatorius,* succeeded by the *vas deferens,* leads to two testes of which the one is outstretched forward and the other backward. Furthermore, so-called bursal muscles are present in the tail end of the male on each side a left hand and a right hand series. They are slightly inclined to the body axis and by their action the tail end of the male is enabled to embrace in a spiral manner the body of the female. Besides these organs there are numerous papillae on the male tail end, being the endings of nerve fibres, as shown in Figures 1 and 2.

Thus the organization of the female *Ag. decaudata,* is distinctly different from that of the male. Hagmeier observed another difference between males and females of *Ag. albicans,* namely, the size of the amphids (Cobb) or lateral organs, which he says are smaller in the female than in the male. In our specimens we were not able to observe this distinction.

The intersexes observed in American *Ag. decaudata* and now about to be described, are, as already mentioned, all



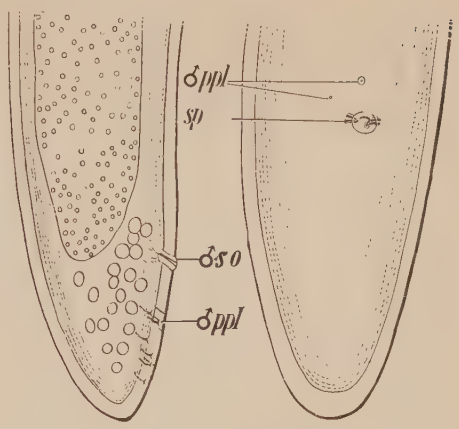
FREE-LIVING LARVAL FORM

FIGURE 4. The first larval stage of *Ag. decaudata* is free-living, while the second larval stage is parasitic upon grasshoppers, roaches and possibly other insects. When the larva enters the host or shortly afterward about five-sixths of the body is dropped and only the head end (*a,* in figure) continues to live and grows into the adult. Division of the body takes place at a preformed node (*n*). This is one of the most remarkable cases of self-amputation known and was first observed by Dr. N. A. Cobb. It shows that the part of the alimentary tract that is homologous with the rectal and anal regions of other nematodes (*r,* in figure), is dropped in this species. Therefore what openings occur at the tail-end of the adults can only be regarded as sex openings and never as anal openings.

females. They all have complete normal female sex organs and most of them normal eggs in the uteri, and all were found encoiled and copulating

TAIL OF INTERSEXUAL FEMALE

FIGURE 5. Two views of the tail of female *Ag. decaudata*, having a male sex opening (♂ *so*). In the normal female no such openings occurs, and this cannot be considered a rudimentary anal opening because that region has been lost in the adult form (see Figure 4). It is difficult to arrange intersexual characters in a very definite graded series because in some cases one male organ will be highly developed and in other individuals this organ will be rudimentary and others will be much more prominent. *c*, sub-median longitudinal chord; *f*, fat body; ♂ *so.*, male sex opening.



MALE CHARACTERS MORE HIGHLY DEVELOPED

FIGURE 6. Tail ends of two females showing more marked intersexuality. The individual on the left has a male sex opening (♂ *so*) and male papillae (♂ *ppl*). The one on the right has two rudimentary spicula (*sp*), and male papillae much less developed than the other specimen. Irregularity in the development of the male organs is characteristic in these intersexes. It is believed that this is due to the fact that these forms are produced by hybridization of different races, in which the time and rate of differentiation of the sex organs vary considerably.

with males. The intersexual state is only to be determined by examination of the tail end. This tail end has more or less the structure and organization of the tail end of a male. We have pictured such transformed female tail ends in Figures 3, 5 to 8. All grades of transformation can be seen from the typical female to a typical male tail end (Figure 2).

Figure 3 is the tail end of a female which shows only a small amount of tissue (connected with the fat body) which has to be regarded as tissue of a male character, since in the normal female no such structure exists.

A more pronounced intersexuality is developed in the female whose tail end is sketched in Figure 5. Here a rudimentary male sex opening is present. It may be said that this structure should be compared with an anal opening and be designated as such. The ancestors of Agamermis certainly once had a complete alimentary tract, and therefore also a functional anal opening. The figured rudimentary openings could be said to be a rudimentary anal opening, homologous with the anal openings of other female nemas, but the ontogenetical development of our forms precludes such a possibility. As Cobb first observed, and as described in the above-mentioned preliminary paper[3], the young larva of *Ag. decaudata* in entering the host loses about five-sixths of its total body length by self-amputation. What develops into the adult *Ag. decaudata* is only the small head part of the preparasitic larva, shown in Figure 4. At a point about one-sixth of the larva's total length from its head end a small series of coin-like cells develops, forming a distinct node. In entering the host the larva drops all of its body posterior to this node by breaking in two at this point. Sometimes the posterior part of the larval body does not enter the host at all, breaking off as soon as the head of the larva has worked its way into the host. In other cases the whole larva enters the host

and the act of self-amputation takes place later.

It follows from these facts that the above-mentioned openings of the female tail end of *Ag. decaudata* and perhaps of other female Mermithids can not be regarded as rudimentary anal openings, but must be sex openings, as all tissues which are homologous with the posterior intestine, the rectum, and the anus of the majority of adult nematodes are dropped by the Agamermis larva when the act of self-amputation takes place.

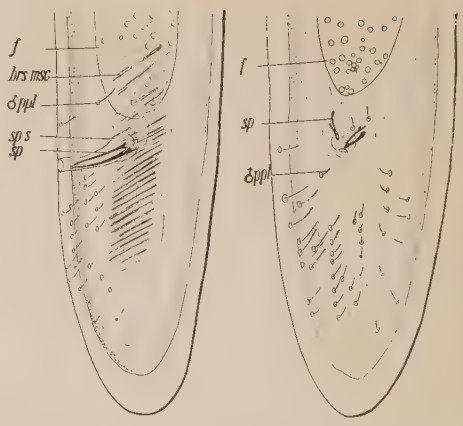
Furthermore the relative frequency of these openings, and the fact that such female tail ends form a graded series linking the normal female and the normal male tail end, speaks against the conception that they are anal openings. If we take this grade of transformation out of the series, the other members of it could not be understood or placed, whereas by regarding it as a reduced male sex opening, it completes a natural structural series that can be understood without difficulty. Hagmeier too regards these openings as rudimentary male sex openings and not as vestiges of an anal opening.

Figure 6 is a sketch of two female tail ends, one of which has not only the male sexual opening, but also a number of male papillae. The other represents a further step to more pronounced intersexuality. There is a rudimentary male sex opening, and also two very small spicula and traces of two male papillae.

Much more pronounced is the intersexuality of the female of which the tail end is pictured in Figure 7. The first gives a lateral, the second a ventro-medial view. A distinct sex opening is present as well as spicula, which are asymmetric; the spiculum of the left side consists of two separated pieces. Some tissue belonging probably to the sheaths of the spicula is also developed. Numerous bursal muscles and male papillae are also present, the latter not fully grown.

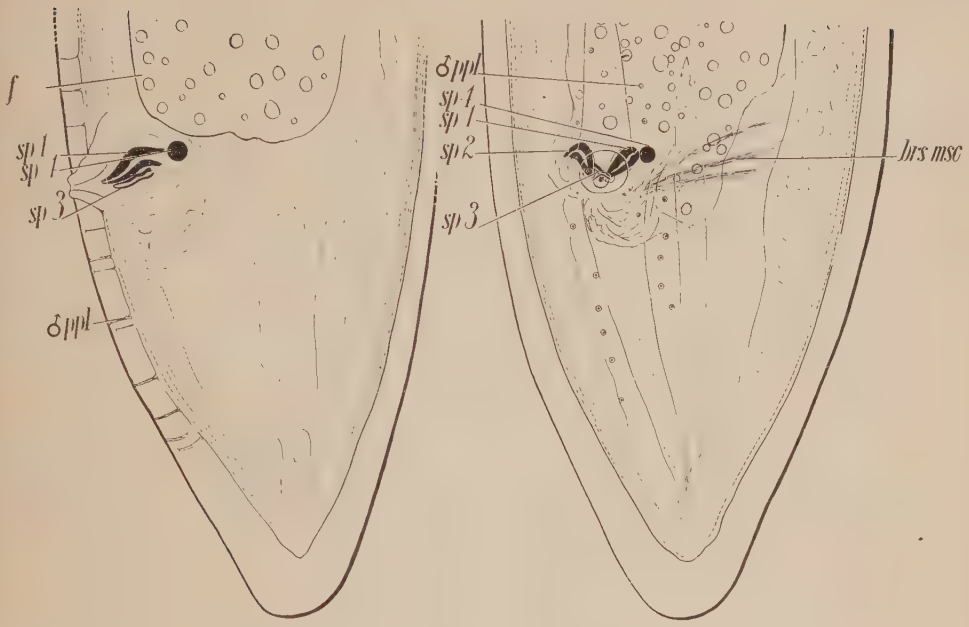
The tail end of an intersexual female sketched in Figure 8 is of particular interest, having four pieces of spicula; moreover, they are asymmetric left to right; those at the left have the general form of spicula, the one being smaller than the other. At the right, there is, besides a somewhat normal-shaped but small spiculum, a globular body. Tissue has developed around the spicula, also a sex opening can be observed as well as some few bursal muscles. Male papillae of more rudimentary character are also present.

Three more advanced cases of intersexuality were mentioned and described by Meissner in *Ag. albicans*. I myself have not yet seen such high grade intersexes. In these three specimens of Meissner even the size seems to have been involved in the transmutation toward the male. He states that all three of these females were dwarfs, compared with normal females, and had only the length of males. It accords with this that the other male characters also were more developed than in the specimens above described. Meissner says that the tail ends of his females were completely masculine, with two normally formed spicula of



WELL-DEVELOPED SPICULA

FIGURE 7. Two views of a female with well developed spicula (*sp*), bursal muscles (*bur musc*) and papillae (♂ *ppl.*). It must be borne in mind that all the individuals shown in these illustrations have in addition to the male organs a complete and functional set of female organs. Left, lateral view; right, medio-dorsal view; *sp s*, spiculum sheath.



TWO VIEWS OF A HIGHLY INTERSEXUAL FEMALE

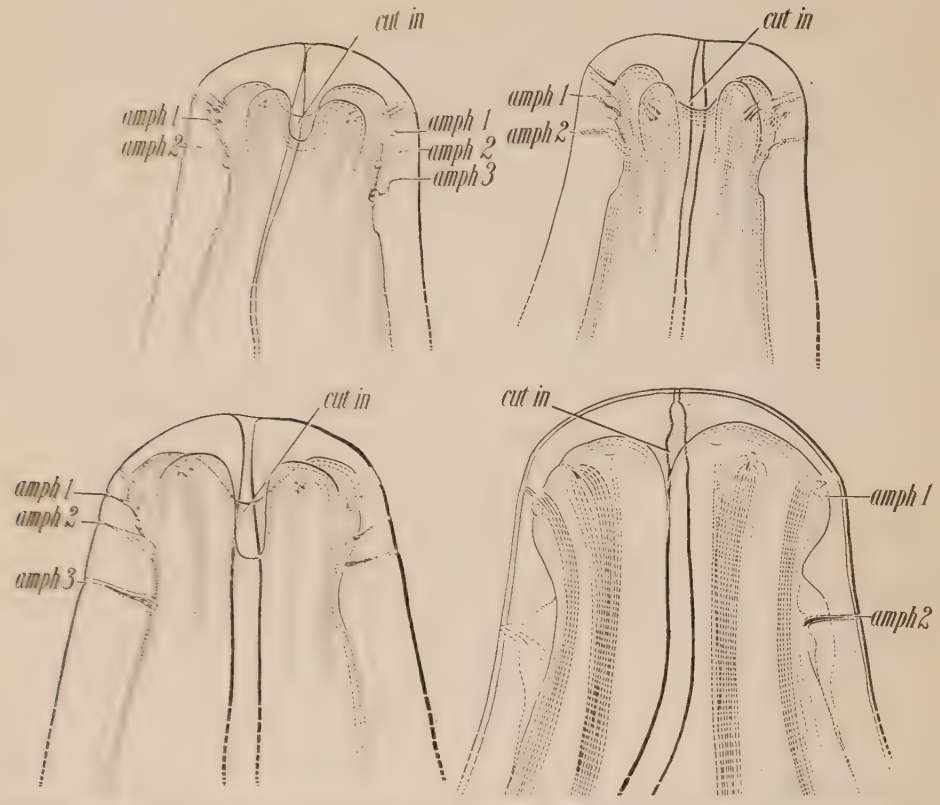
FIGURE 8. This female has a double set of male spicula (*sp* 1-4). Three are normal in shape, and the fourth is represented by a spherical body. Notice that there are much fewer papillae than in the female shown in Figure 7. These intersexes are probably due to natural crossing of distinct races or closely related species, as were the intersexes of the gypsy moth produced artificially by Goldschmidt. Left, lateral view; right, ventra-submedial view; *f*, fat body; *δ ppl*, male papilla; *sp* 1, spiculum of the left side; *sp* 3, second spiculum of the right side; *sp* 4, globular second spiculum of the left side; *burs musc*, bursal muscle.

equal length and thickness with the sheaths well developed, but the muscles around the spicula were not as strong as in the true male. However, the bursal muscles, the rows of male papillae, and even the general form of the tail end was exactly like the normal male. But there was no trace of male gonads and ducts. Very interesting it seems to me is Meissner's statement that in the intersexual females the eggs were, though normal, somewhat smaller.

Undoubtedly these intersexes are the most pronounced of this kind hitherto observed in Mermithids. They allow us to construct a complete series of intersexual females with all possible grades between a normal female and a female with complete male secondary sex characters.

As already stated, nearly every investigator who has studied carefully adequate material of *Ag. albicans* has observed such intersexes. Intersexuality seems in this species of *Agamermis* to be a rather common phenomenon. *As far as we know there is no other group of animals known today in which intersexes are observed in such numbers under natural conditions and normally produced.* This seems to be a fact specially to be noted.

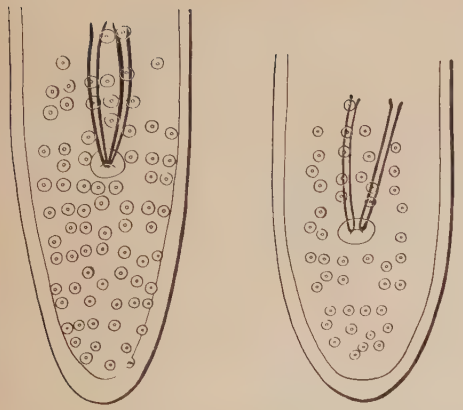
What are the causes of these conditions? As yet we do not know, but there are some other facts about *Agamermis decaudata* and related forms which may indicate where we must look for an explanation. Recently intersexes have been produced experimentally by several investigators, as by Goldschmidt in *Lymantria dispar;* by Keilin and Nuttal in *Pediculus capitis* and *P. corporis;* by Sexton and Huxley in *Gammarus chevreuxi* and related



VARIATIONS IN HEAD ORGANS IN SPECIES PRODUCING INTERSEXES

FIGURE 9. The head organs of most species of nematodes are very constant in form and position. The function of the amphids (*Amph* 1-3) is not definitely known, but there is reason to believe that they are chemical sense organs. The fact that they are so similar on different individuals of the same species makes them of great value in the systematic classification of nematodes. In the case of *Ag. decaudata,* however, we find a striking exception to this rule, for great variation is observed in the number and position of the amphids. This is further evidence of the existence of numerous closely related species which intercross in nature. *Amph.* amphid; *cut in,* cuticular incision between the papillae of the left and the right side.

forms, by Bridges in mutants of *Drosophila melanogaster* and by Sturtevant in *Drosophila simulans.* In all these cases intersexes resulted from hybridization of closely related genotypes. Are there signs that also in our case the observed intersexes could be the result of hybridization of races or genotypes closely related and living mixed with each other in nature? This appears to be the fact. Many difficulties arose as I tried to identify the material brought to me. In the taxonomy of nematodes the structure of the head end and chiefly the organization, the location and number of the head sense-organs are of high importance. This is particularly true in the Mermithids, where other characters for the distinction of the species are very few in number and many of them to be seen with great difficulty or only on adult specimens. Therefore, a close study of the head end and its sense-organs is absolutely needed for a good identification. There are two kinds of head sense-organs in most of the nematodes—the *papillae,* which are claimed to be organs of touch, and the *amphids* (Cobb) or *lateral organs,* which are



TAIL ENDS OF NORMAL MALES

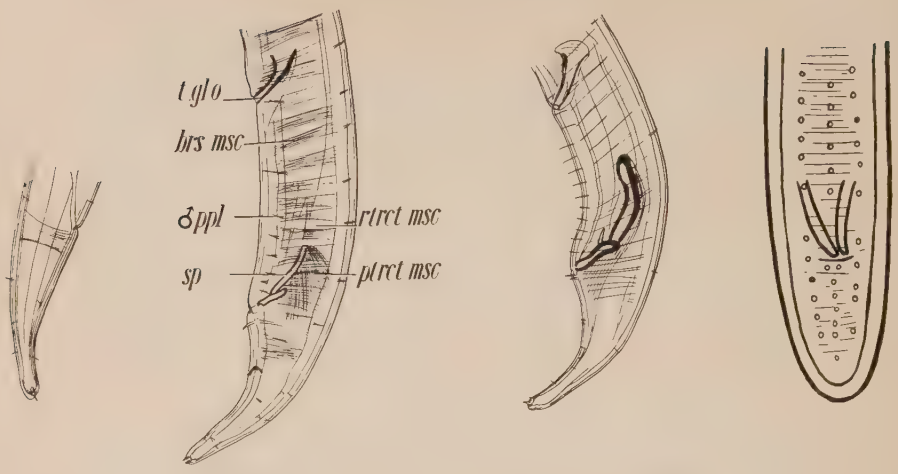
FIGURE 10. In addition to the variation in the head organs of this species, other normally constant characters are unusually variable. This shows the tail ends of two normal males of *Ag. decaudata.* Note the considerable variation in the position and number of the papillae. These differences are greater than are found in other species of nematodes.

probably chemical sense organs. These organs are of very constant nature in nearly all nematode species, and the amphids, or lateral organs are especially of high value as specific characters. In the Mermithidae this is particularly true. The adult specimens may be identified more or less easily by the aid of the sex organs, but the larval specimens can only or best be distinguished by the sense organs of the head. The position, the size, and the structure of the amphids is very different in different species of Mermithidae, although within the species, with few exceptions, very constant. Such exceptions are *Ag. decaudata* and related forms. The number, the structure and the position of the amphids varies here very much, but other significant and easily perceptible differences have not yet been discovered. There are differences also in the shape of the tail end, but these are insignificant. Perhaps the number of metabolic cells along the esophageal tube may be of taxonomic use if more were known about them.

Figure 9 shows the head ends of specimens of *Ag. decaudata,* all differing from each other in respect to the amphids. Why in this form are the amphids so inconstant, while they are so constant in most of the other nematodes? Is it not that we have in *Ag. decaudata* and related forms, not sharp-cut species, but an association of different races or genotypes and numbers of hybrids between them? These races are perhaps continually crossing and it is thus that such a multitude of structural forms in the head end comes about. The numerous intersexes are another result of these crossings between closely related genotypes. In the light of the experiments of Goldschmidt and the other above-mentioned investigators, where intersexes resulted from hybridization of closely related forms, it is highly probable that there is a causative relationship in this case between the numerous related species and races, and the number of intersexes observed. Yet this is only an explanation which seems to give most satisfaction, and could not be accepted as final until the matter has been tested by experiment.

More recent experiments point to the same conclusion. What was formerly called *Mermis albicans* by different authors is perhaps a group of morphologically closely related forms. It may be that they are species, or at least races. One of these may perhaps be our *Agamermis decaudata,* which in the adult stage resembles to a high degree the former species and was apparently taken as such by Leidy and other authors. Experiments by T. R. Christie and the writer show that the larvae of this latter species (decaudata), could not be brought to infest other animals than grasshoppers and roaches. Yet this form very much resembles the former, which is said by many writers to parasitize insects of the most various groups, and even snails.

All these facts, it seems, are good evidence that the numerous intersexes observed as normally forthcoming in



INTERSEXES FOUND IN OTHER GROUPS

FIGURE 11. Intersexes have also been observed in several other groups of nematodes. The three left hand figures show respectively the tails of a normal female, a highly intersexual female, and of a normal male of *Enoplus michaelsoni* v. List. (After de Man). This form is a free living marine nematode, very different from the species discussed in this paper. On the right is shown the tail of a highly intersexual female of *Mermis mirabilis*, as described and figured by von Linstow. The male copulatory organs of this female were well developed. *burs musc*, bursal muscle; *♂ ppl*, male papilla of bristle shape; *ptrct mus*, protractor muscle of the spiculum; *rtrct msc*, retractor muscle; *t gl o*, tubular gland outlet, typical of the male; *sp*, spiculum.

our Mermithids are a result of hybridization.

Intersexes in Other Mermithid Species

Ag. decaudata and *Ag. albicans* are far from being the only Mermithid species where intersexes have been observed.

v. Linstow mentions (1903) an intersexual female in his *M. mirabilis*. In this specimen the female sexual organs were well developed, but on the tail end male copulatory organs were to be seen; a male gonad was absent (Figure 11).

Hagmeier observed intersexes in what he terms *Mermis terricola* Hag. Among twelve specimens two were intersexes (Hagmeier calls them hermaphrodites); both had the appearance and size of females; ovaries, uteri, vagina and vulva were well developed; in the uterus of one, ripe eggs were to be seen. But the tail was completely masculine. The spicula of one particularly large specimen were even larger than those of a normal male, but not so strongly, and somewhat irregularly bent, with knot-like thickenings. The sheaths of the spicula and also the long *retractores spiculorum* muscles were present, whereas the bursal muscles were present only in the tail end. The male papillae were typically formed.

The second specimen showed more irregular spicula and only a few male papillae, but here the bursal muscles were fully developed, even also orad of the male sexual opening, but in both cases Hagmeier was unable to find any traces of male gonads.

The same author observed also isolated bursal muscles in some female specimens of his *M. elegans*, *M. albicans* and *M. arsenoides*. Male sexual openings were often observed by him in females of *M. albicans*, but sometimes also in *M. elegans*, *M. arenicola* and *Paramermis fluviatilis*. Unfortunately we do not know the conditions here accurately enough to judge whether the above outlined explanations would fit. Further investigations will have to be

made before the matter will be cleared up. From my own observations I believe that variations similar to those described above for *Ag. decaudata* and related forms may be found in other Mermithid species.

Exactly why the Mermithids produce such an abundance of intersexes, is still unknown, and also why, apparently, only females show intersexual characters. Hitherto no one has observed, or at any rate mentioned, intersexual males in this family. In regard to *Ag. decaudata* it may, however, be mentioned that the number of the papillae on the tail end of the male is extremely variable, as is the arrangement also. It is hard to find even two specimens which are in this regard perfectly alike; also the length and even the form of the tail itself is somewhat variable as well as the length of the spicula (Figure 10).

Intersexes in Free-Living Nematodes

Apart from the family of the Mermithidae intersexes have been seen in very few other nematode genera. Somewhat outstanding in this regard is the genus *Trilobus,* including a number of fresh-water free-living forms. In 1905 (l. c. p. 55) E. von Daday describes a very pronounced case of intersexuality in *Trilobus longus* Leidy (*Trilobus diversi-papillatus* v. Daday.) It was an intersexual female. The ovaries, uteri and vagina of this female were well and normally developed, the vulva had its normal position and eggs were seen within the uteri. But this female had also well developed spicula, and, according to v. Daday, all the preanal accessory organs of the male, consisting of a group of three large, three medium-sized and numerous small preanal papillae. He claims even to have seen a short testis with a short *ductus ejaculatorius,* but apparently not functioning.

More often intersexes have been observed in *Trilobus gracilis* Bastian. Of this species Ditlevsen mentions a female with well developed male papillae, such as characterize the male, but without spicula and gubernacula. Recently Wilhelm Schneider observed two intersexual females of the same species. One had completely developed spicula but no trace of any papillae, the other female showed only three preanal papillae, whereas the spicula apparently were absent. Both specimens had well developed female organs. Dr. N. A. Cobb tells me that he also has observed on several occasions intersexual females in *Trilobus* at Washington, D. C., and Mr. Thorne tells me the same thing for specimens living in fresh water from Utah.

Regarding the causes of the intersexuality in this group of free-living nemas, it is of interest that, at least in *Trilobus gracilis Bastian,* there exist a series of races, genotypes or varieties already mentioned by several authors. I refer in this regard to the paper of Stefanski (1917), and one published by the writer (Steiner, 1919). Undoubtedly we have here a whole series of closely related, but slightly different forms, probably not all known. *Here, too, the observed intersexes may be related with hybridization between these different genotypes.* The abovementioned *Trilobus longus* is regarded by Micoletzky (1921) as only a variety of *T. gracilis,* but further studies have yet to prove the correctness of this conception.

Intersexes in Marine Free-Living Nemas

J. G. de Man mentioned in 1893 a thoroughly functional female of *Thoracostoma figuratum* Bast. with the spicula, and the gubernaculum of a male and a similar specimen of a female of *Chromadora poecilosoma* de Man.

In 1904 the same author mentions another very interesting case. Of nine specimens of *Enoplus michaelseni* v. Linst. found in algae growing on Gasteropods in the Bay du Torrent, Isle of Londonderry, Canal francais, Antarctic, he observed three females (they were the largest in size), with very pronounced intersexual characters. Two of them had eggs in the uteri; all three had the preanal, ventro-medial cutinized gland outlet of the male and also a

series of fine hairs between this outlet and the anus as in the normal male. In regard to the spicular apparatus, the three specimens represented three transitional grades; in one the apparatus was very similar to that in the normal male. In the third female, however, it was totally different. (See Figure 11.)

Comparing the intersexes of the different species described above, it is very remarkable to note that the different organs involved in the transformation toward the male sex are not progressive. As in the intersex represented in Figure 7, the bursal muscles and the papillae are more developed than in the one sketched in Figure 8, where the spicula are of a more advanced type.

Furthermore, comparing other cases mentioned in this paper regarding this character (f. c., also Figure 6), there are more such irregularities. It seems as if there is no correlation tending to produce gradual stages of development of the different sex organs. Where in one specimen an organ may proceed far along in its transformation, in another it may remain much less developed when compared with other organs involved in the latter transformation. In a recent paper by Goldschmidt (1922), which has just come to my attention, he presents an explanation of similar cases in his broods of *Lymantria dispar*. This explanation may also be applied here. He states that the races of *Lymantria dispar* show a great diversity in the rate of their development, and also in regard to the initiation of differentiation of the various organs which are known to occur at different intervals of time. Inasmuch as these characters are heritable, the hybrids consequently also show differences in this respect. The variations in the rate and size of development of the intersexual organs are a result of these conditions.

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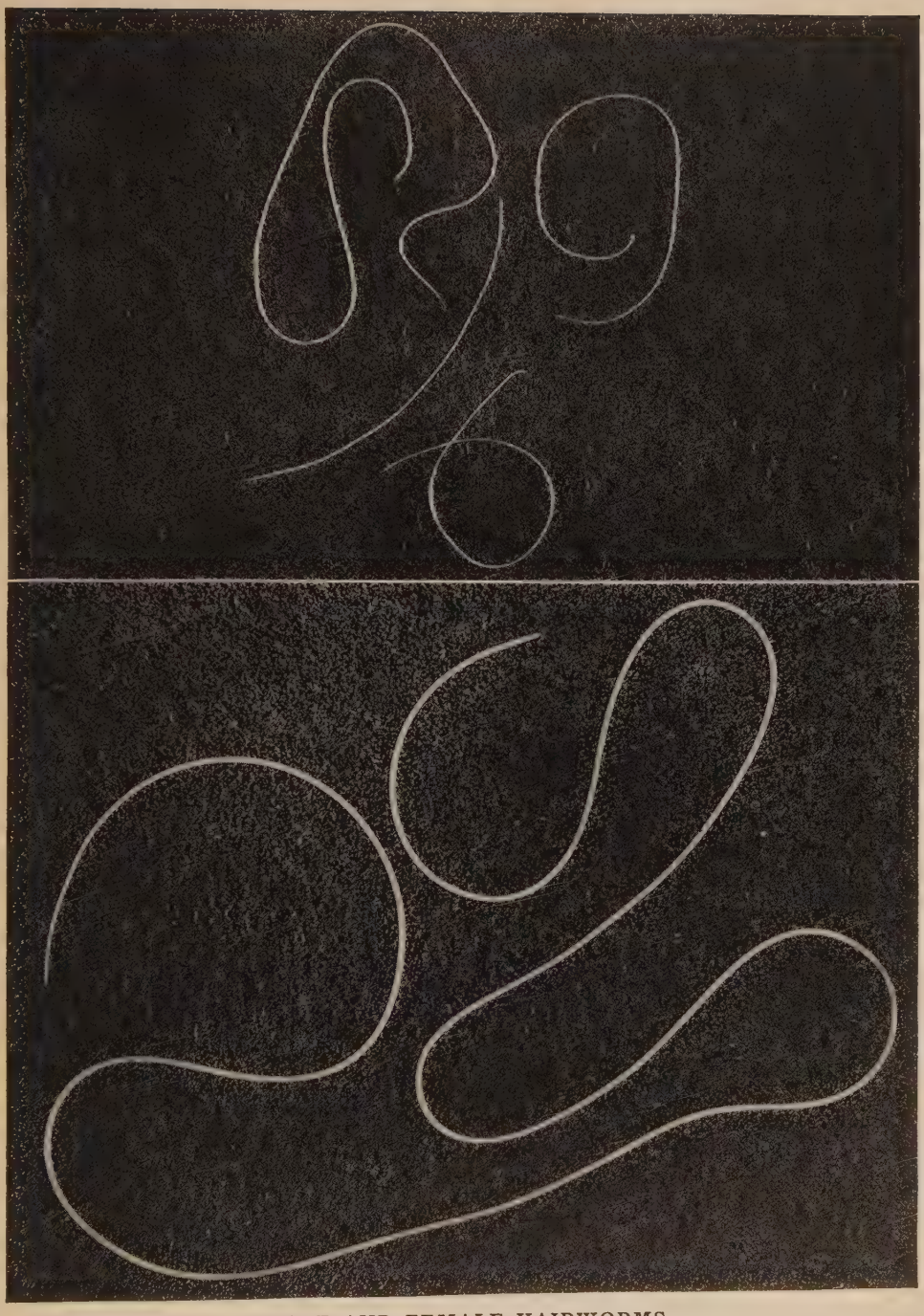

MALE AND FEMALE HAIRWORMS

The genus Agameris contains some of the larger species of hairworms. The average length of the females of *Ag. decaudata* (bottom) is nearly eleven inches; that of the males (top) is not quite three inches. Many nematodes are parasites, and nearly every kind of plant and animal is an actual or potential host for one or more species of them. Nematodes are not immune from attacks by their own kind; in some cases they even prey on individuals of the same species, something very unusual among anything but human parasites. Their parasitic habit gives nematodes great economic importance. Some species are a serious menace to crops and herds, while others prey on our most dangerous insect pests and offer a promising means of combating them.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, OCTOBER 8

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THE CONNECTING SYSTEMS OF THE REPTILE HEART—ALLIGATOR

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FOUR FIGURES

The connections between the divisions of the reptile heart along which the impulses for contraction are transmitted have been described by a number of investigators. Inasmuch as adequate summaries of the literature are given in the papers of Laurens ('13 a and b, '15, '16), Mangold ('14), and others, no detailed review will be repeated here. The Crocodilia, however, have been somewhat neglected in this respect, although Mackenzie ('13) deals briefly with the connecting systems in the heart of the crocodile. The Crocodilia are phylogenetically the lowest group of vertebrates possessing a complete interventricular septum and might be expected, therefore, to represent a transitional stage between the simple auriculo-ventricular funnel of the lower reptiles, amphibia, and fish, and the much reduced, though more complex, bundle of mammals. Young alligators are readily available for physiological experiments, and this account is intended not only as a record of the morphology of one more auriculo-ventricular connection, but also a somewhat diagrammatic background for physiological work on the heart.

[1] This study was begun and in large measure carried out at the Osborn Zoological Laboratory, Yale University, and the author wishes to express his thanks to Dr. Henry Laurens both for suggesting the problem and for valuable criticism and advice. The writer is also indebted to Dr. George L. Streeter for permission to use the facilities of the Carnegie Institution of Embryology in the construction of the model.

Gross dissections of fresh and preserved specimens (18 inch) were made to trace out the nerves which supply the heart musculature. The sections used were from hearts fixed by injection of corrosive acetic, cut serially 10 μ thick in transverse, sagittal, and frontal planes, stained with iron haematoxylin and in some cases counterstained in eosin.

ANATOMY AND INNERVATION

The heart of the alligator has been described before (see Bronn's Thierreich for summary and literature) and it only remains, in view of the scope of the present work, to consider briefly the features that bear directly on the subject at hand. A very striking feature is the size of the bulbus (fig. 1) which is made up of the proximal part of the arteries leaving the heart and is as large as all the rest of the heart together. The auricles are approximately equal in size, the right extending further ventrally around the side of the ventricles than the left. The sinus is of considerable size, somewhat triangular in shape and receives six veins which open into it separately. The two superior cavae, right and left, drain into the corresponding cephalic angles; the hepatic veins from the right

ABBREVIATIONS

AVB, auriculo-ventricular bundle
B, bulbus arteriosus
DL, dorsal ligament
GTV, ganglion trunci vagi
IVS, interventricular septum
L, margin of lung
LA, left auricle
LAO, left aorta
LC, left carotid
LV, left ventricle
LVA, left vertebral artery
LVC, left superior vena cava
LVV, left vertebral vein
RA, right auricle
RAO, right aorta
RV, right ventricle
RVA, right vertebral artery
RVC, right superior cava
RVV, right vertebral vein
S, sinus venosus
SNB, sympathetic nerves to bulbus
SNS, sympathetic nerves to sinus
T, trachea
V, ventricle
W, portion of auriculo-ventricular bundle in wall of right ventricle
X, portion of auriculo-ventricular bundle in interventricular septum
Y, portion of auriculo-ventricular bundle in wall of left ventricle
Z, fine connection about 20 μ wide between bulbus musculature and left auriculo-ventricular bundle

and left lobes of the liver open at slightly different levels further posteriorly; the inferior cava connects with the caudal angle; and the coronary reaches the sinus about the middle

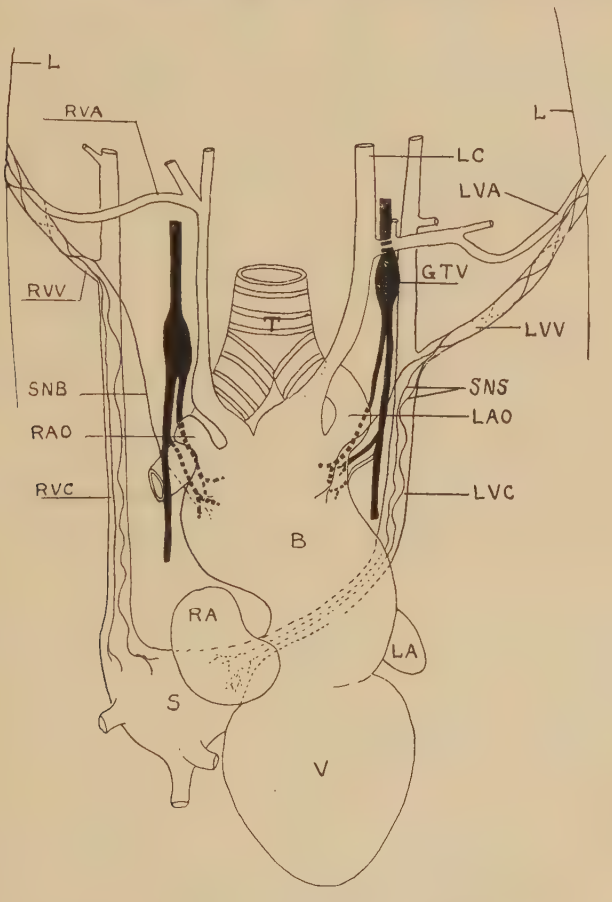

Fig. 1 Diagrammatic ventral view of alligator heart, to show the course of the vagi and sympathetic nerves to the heart.

of its ventral surface. This latter vein comes from the dorsal wall of the ventricle via the dorsal ligament of the heart—a structure which will be considered more fully later.

The innervation has been described by Gaskell and Gadow ('84). Figure 1 shows in a diagrammatic manner how the

nerves reach the heart. From the sympathetic ganglion of the 11th metamere, which is larger than any of the others, two postganglionic branches of considerable size are given off in addition to the usual filaments. These were traced by dissection and found to pass from their ganglion of origin ('G. cardiacum,' Gaskell and Gadow) to the surface of the vertebral vessels which they follow over the latero-ventral surface of the lung. The fibers are small and anastomose and rebranch freely around the vessels up to the junction of the vertebral vein with the vena cava. At this point the nervous elements resolve themselves into two branches, one of which immediately divides again and follows the cava to the heart. These branches are very small and closely applied to the vein. A second branch follows the vein for some distance, but at the level of the emergence of the pulmonary artery from beneath the arch of the aorta, it veers medially and sends a branch along each side of the pulmonary artery to end in the walls of the bulbus. During the course of the nerves from the ganglion to the heart no communication with the vagus could be detected. The superficial course of these nerves diagonally over the lateral and ventral surfaces of the lungs in company with the vertebral vessels makes them easily accessible for manipulation in physiological investigation.

The vagus, shortly before reaching the level of the bulbus, bears an ovoid enlargement, the ganglion trunci vagi. This ganglion is situated at a slightly more posterior level in the case of the right vagus. From it a well-defined branch is given off which passes caudally to the space between the pulmonary artery and the aorta where it turns to spread out on the dorsal aspect of the bulbus. The main trunk of the nerve crosses the dorsal side of the pulmonary artery, gives off a variable number of branches to the bulbus and the lungs and proceeds posteriorly down the dorsal wall of the abdominal cavity. The ultimate distribution of these nerves could not be determined by gross dissection, but in sections nerve branches with groups of ganglion cells along their course were found

widely distributed in the epicardium, supplying all parts of the heart.

THE SINO-VENTRICULAR CONNECTION (THE DORSAL LIGAMENT)

A discussion of the anatomy of this structure has recently been given by Laurens ('15), who considered its nature in different forms with a historical review of the question. In agreement with Laurens' description for the lower reptiles, it consists in the alligator of a more or less cylindrical fold of the pericardium which separates itself from the wall of the sinus about the middle of its ventral surface and passes ventro-cephalad to the auriculo-ventricular groove passing free over the dorsal surface of the right auricle. At its points of connection with the sinus and ventricle it broadens slightly into a small fan-shaped structure, becoming immediately indistinguishable from the surrounding pericardium. Examination of this structure in the living heart reveals the fact that it transmits the coronary vein from the auriculo-ventricular groove to the sinus. Histological study readily shows that, in addition to the coronary vein, there are included in this ligament a more or less variable number of nerve branches ('coronary nerve,' Gaskell, '84) and a small artery. Both Gaskell and Laurens have noted that the nerves in the dorsal ligament are sometimes insignificant or even lacking. These nerves and blood vessels may easily be made out in figure 2, which shows a transverse section through the heart in the region of the attachment of the ligament to the ventricle. Here the dorsal ligament is shown as a conical projection from the pericardium and continuous with it at a number of points. The appearance of the ligament in sagittal section is shown in figure 3, in which it is seen connected with both sinus and ventricle. The areolar connective tissue in which the nerves and blood vessels are imbedded is similar to and continuous with that under the pericardium in all regions. Ganglion cells in profusion are also contained in the ligament as elsewhere under the pericardium, but no muscular fibers are present

and the structure cannot therefore be considered as part of the coordinating mechanism of the heart.

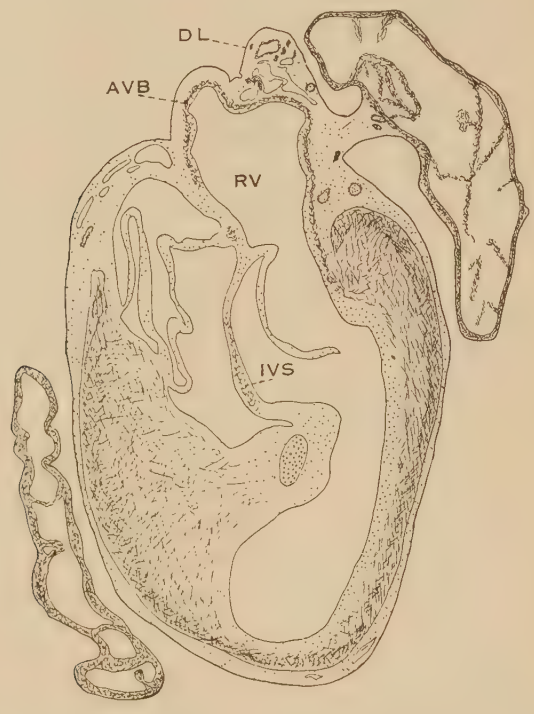

Fig. 2 Transverse section of the heart at the level of the attachment of the dorsal ligament to the ventricle. The interventricular septum is for the most part membranous; the auriculo-ventricular bundle is seen extending into it on the dorsal side. × 15.

THE SINO-AURICULAR CONNECTION

As in the reptile hearts studied by other workers, particularly those described by Laurens ('15), the connection between the sinus venosus and the right auricle in the alligator is a direct muscular one. The sino-auricular opening is situated close to the auricular septum, the right valve is a fold of the sinus wall, the left a combination of sinus wall and septum. The muscular fibers from the sinus are somewhat invaginated into the ventricle, thus simulating the auriculo-ventricular

connection. Both valves contain muscular fibers which in the right or lower valve, go over directly into the fibers of the auricle, while in the left they pass also in part to the septum. A considerable muscle bundle passes by the base of the left valve without taking part in its formation and goes directly

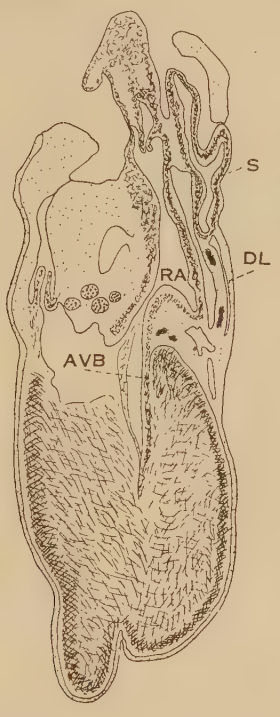

Fig. 3 Sagittal section of the heart, showing the dorsal ligament and the descent of the auriculo-ventricular bundle into the right ventricle.

to the auricular wall and septum. No specialized bundle could be made out nor were any histological differences noted in the musculature of these portions of the heart. In the connective tissue surrounding this region and interspersed between the muscular fibers many capillaries, nerves, and some ganglion cells were found.

According to Mackenzie ('13) the sino-auricular muscle, or its remnant, in the crocodile is present at the base of the left

venous valve at the junction of the sinus with the septum, there being no direct continuity between the sino-auricular and the auriculo-ventricular bundles, as in the South American lizard, Salempenter. Mackenzie describes the interruption between the two as taking place in the region of the sinus septum where the left duct of Cuvier enters, the sino-auricular nodal tissue appearing to become lost in the septum, which grows forward from the posterior wall of the sinus.

Laurens ('15) called attention to the fact that the conditions found in the lizard and tortoise hearts which he had studied placed them as intermediate stages between the conditions found in the iguana and in the crocodile. From the results of the present study we would place the alligator in the same group with the lizards and tortoises studied by Laurens.

THE AURICULO-VENTRICULAR CONNECTION

As has been shown by Mackenzie ('13), Külbs and Lange ('10), Külbs ('12, '13), and others, the auriculo-ventricular connection in different groups of animals varies all the way from the simple muscular ring of the fish heart to the specialized and relatively complicated condition found in the mammal. In a general review by Mangold ('14), the changes, progressive and regressive, in the auriculo-ventricular connection of the fish, amphibian, reptile, and bird are clearly discussed.

Mackenzie believes that the bundle in the crocodile, as in the mammal, descends from the auricle behind and beneath the membranous septum, the heart being completely divided. The interventricular septum, as in the mammal, he regards as (p. 138) "composed of three parts: (1) an anterior, derived from the bulbo-ventricular ridge; (2) a posterior, growing forwards and upwards from the interventricular raphe; and (3) between these the membranous part of the septum."

I have found that in the alligator the interauricular septum is directly continuous with the interventricular. Its muscular

portion goes over into the muscular part of the interventricular septum posteriorly and its membranous caudal border is continuous with the adjacent part of the interventricular septum. According to Mackenzie (p. 130), "there is no descent of auricular canal fibers into the left ventricle in the case of the crocodile. The fibers which originally descended

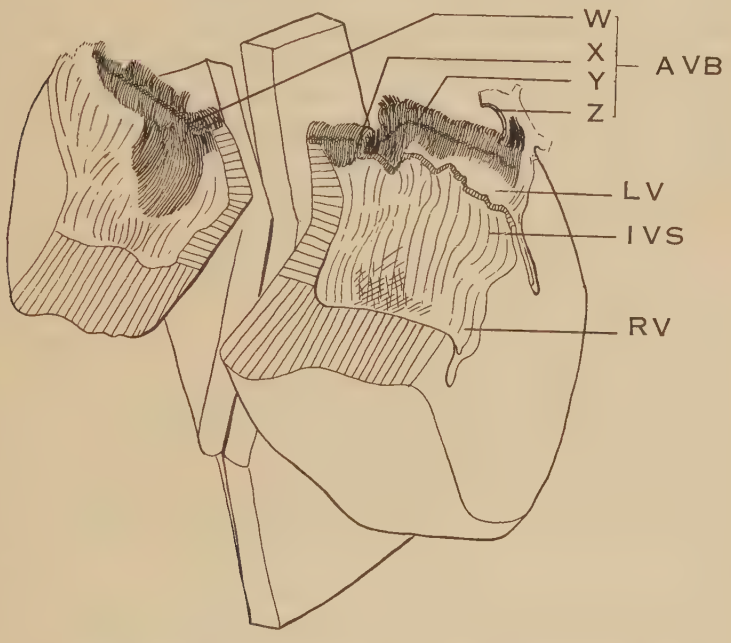

Fig. 4 Drawn from a photograph of a wax-plate reconstruction model of the heart, made from serial frontal sections. The membranous part of the interventricular septum is not shown and the auricles have been removed. × 6.

into the left ventricle have become atrophied by the development of the parietal auriculo-ventricular valve on this side."

In the alligator the auriculo-ventricular bundle has not undergone such marked reduction as is described by Mackenzie for the crocodile. As shown by the model (fig. 4, cf. also figs. 2 and 3), there is a considerable invagination of auricular fibers into the lateral wall of the right ventricle and into the posterior part of the interventricular septum, while in the

left ventricle the bundle, though not so strong as in the right, nevertheless is present as a definite though interrupted sheet of fibers. The connection in the alligator differs from the condition found in the hearts of the lower reptiles, however, in not being a complete funnel or ring on account of the interposition of the membranous bulbus on the ventral side. A narrow tongue-like band projects ventrally into the interventricular septum from the posterior wall of the partial funnel, giving the bundle somewhat the appearance of an 'M' in optical cross-section. The sheet into the right ventricle is quite strong and descends for a considerable distance before merging with the ventricular musculature. In the left ventricle, at the base of the parietal valve, the sheet of descending auricular fibers is interrupted by connective tissue and even between the valves it does not extend far into the ventricle.

In its finer structure the connecting tissue resembles the musculature of the auricles very closely. At the region of the auriculo-ventricular openings the fibers are arranged in a more or less circular fashion, but lower down in the ventricle they become directed longitudinally. The distribution to and the intermingling with the ventricular fibers is easily observed, inasmuch as the striations of the latter are much wider and take the stain more deeply. No nerve fibers or ganglion cells could be found in the bundle, although many were seen in the auriculo-ventricular groove and traced to points in the connective tissue very close to the conducting bundle.

Attention should be called to the similarity between the conditions found by Mackenzie in the crocodile and bird heart as contrasted with my findings on the alligator. Mackenzie found that in crocodiles and in birds, in addition to occasional strands of muscular connection over the free edge of the muscular valve, there is a distinct bundle of nervous and muscular tissue which descends from auricle to ventricle at the posterior part of the interventricular septum, and in the angle where the muscular and septal valves are adjacent. There is no descent of auricular canal fibers into the left ventricle.

Mangold and Kato ('14) by experimental means have confirmed Mackenzie's description for the bird. They find, however, a connection for the left heart, which originates in common with that for the right, from which it divides either in or just above the auriculo-ventricular groove to be continued downward after going through the septum.

No doubt, experiments on the crocodile heart would confirm Mackenzie's description for that animal. The conditions in the alligator, however, are different, and I am able to state that experiments which have been carried out in Doctor Laurens' laboratory on the hearts of young alligators have substantiated the above anatomical description of the course of the auriculo-ventricular connection.

SUMMARY AND CONCLUSIONS

1. The heart of the alligator receives nerve branches from the vagus through the ganglion trunci vagi, and from the sympathetic through the ganglion (G. cardiacum) of the 11th metamere. The former reach their destination in a number of small branches and the latter accompany first the vertebral vessels and then the superior cavae to the heart.

2. The sino-ventricular connection (dorsal ligament) is composed of a fold of the pericardium containing connective tissue, blood vessels, and nerves, but no muscle.

3. The sino-auricular connection is a ring of cardiac muscle which surrounds the opening from the sinus into the right auricle and proceeds in part around the margins of the valves which guard this opening.

4. The auriculo-ventricular connection is a trough-shaped bundle of auricular muscle which extends down into the ventricles. It has a narrow tongue-like projection which establishes a connection between the auricles and the interventricular septum on the dorsal side of the heart. The portion of the bundle in relation with the right ventricle is much more extensive than that in the left ventricle, where the interposition of connective tissue at the base of the parietal valve

partially interrupts the connection in many places. Ventrally the membranous wall of the bulbus prevents all connection between auricles and ventricles. There is a fine connection between the bulbus and the left branch of the auriculo-ventricular bundle.

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WEIGHT CHANGES AND OXYGEN CONSUMPTION DURING LONG EXPOSURE TO DILUTE ANESTHETICS

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FIVE FIGURES

INTRODUCTION

In the course of an investigation of the factors concerned in determining head formation in pieces of Planaria dorotocephala regenerating in non-lethal solutions of certain anesthetics, it was necessary to record the oxygen consumption and weights of the pieces, both in dilute anesthetics and in water, at intervals over periods of time varying in different experiments from twenty-four hours to two weeks. For purposes of comparison of animals and pieces of different physiological condition, the results were calculated on the basis of the amount of oxygen consumed during a standard period of time, two hours, per milligram. The changes in oxygen consumption per two hours per milligram, in relation to weight changes, when compared with controls, show some differences during exposure to different anesthetics. The present paper constitutes a record of observations and data obtained in these experiments and briefly summarized elsewhere (Buchanan, '22 d), together with data obtained in subsequent work on intact animals starving under similar conditions, undertaken with the object of extending the investigation. This species is no longer available to the writer in numbers sufficient to permit further studies of this nature.

THE PROBLEM

Although disagreement as to the nature of the action of anesthetics prevails, much evidence has accumulated to show that dilute concentrations of many anesthetics in some way stimulate the activity of organisms, the stimulation being manifested by increased motor activity, increase in rate of growth, increased irritability, and in other ways. (For instances of such observations see Czapek, '13, p. 143; R. S. Lillie, '15; Winterstein, '19, p. 17.) In fact, agreement is general that, while relatively high concentrations inhibit, low concentrations stimulate such processes, for a time at least. Considerable evidence has also been advanced to show that low concentrations of many anesthetics induce increases in respiratory rate in both plant and animal tissues. The evidence varies greatly in detail, however, and, so far as the writer has been able to find, the data do not contain evidence of changes in weight during the period of increased respiratory activity.

Elfving ('86) showed that atmospheres both of ether and of chloroform increased the total carbon dioxide production of Salix leaves over periods of fifteen to twenty-four hours. Similar concentrations had similar effects on Pisum seeds. His data do not show possible fluctuations during the period of exposure. He regards the increase of carbon dioxide output as not resulting from the oxidation of the ether and chloroform. Kosinski ('02) found that the carbon dioxide production of Aspergillus niger increased gradually for a number of hours in environments of 0.2 per cent and 0.25 per cent ether, but he gives no data on possible changes in the organism. Gerber ('02) found respiration in the Banana increased, over twenty-four hour periods, by ether vapor. Zaleski ('04) found that certain concentrations of ethyl alcohol increased the carbon dioxide production of the bulb of Gladiolus, but that it decreased during the later period of exposure. It was shown by Baer and Meyerstein ('10) that the formation of acetone by oxidation of oxybutyric acid in the perfused liver of the dog was increased by trichlorethyl alcohol and other substances. Their data show considerable variations; the

agents were applied for very brief periods, and the results probably represent initial effects only. They record that Lussana and Roli found that ether increased the carbon dioxide production of liver and muscle tissue, while chloroform and chloral hydrate depressed it.

Irving ('11) in a study of the effects of chloroform on the carbon dioxide production in barley shoots and laurel leaves, found that continuous treatment with a small quantity of chloroform vaporized in an air current induced an increase in carbon dioxide production which was maintained throughout the period of exposure (eighteen hours). Her experiments appear to have been very carefully controlled, but deal more particularly with the effects of stronger solutions. Vernon ('12) found that the respiration of the perfused kidney was increased by the addition of certain weak solutions of alcohol. Winterstein ('14) showed that respiration in the spinal cord of the frog was increased during alcohol narcosis. Both Vernon and Winterstein employed periods of exposure limited to several hours, and their data do not in any way show the possible effects of the increased respiratory rate upon the weight of living substance. Tashiro and Adams ('14) found that carbon dioxide production of the claw nerve of the Spider crab increased after short exposure to 0.4 per cent chloral hydrate. They record no change in weight. A fifty per cent gain in weight is noted after like exposure to a completely narcotizing solution (4 per cent). Irwin ('19) showed that high concentrations of ether increased the carbon dioxide production and oxygen consumption of the petals of Salvia. Her data probably represent only the initial effects of lethal solutions, since the maximum exposure was but thirty-five minutes and the concentrations employed were relatively strong. Medes and McClendon ('20) showed that the oxygen consumption and carbon dioxide production of Elodea was increased over twenty-four hour periods by 3 per cent alcohol, 1.5 per cent ether, 0.05 per cent chloroform, and 0.05 per cent chloretone. They record some diffusion of chlorides in these solutions, and also slight decreases in size of the chloroplastids. Recently Bodine ('22) found that small doses of certain anesthetics induced increased carbon diox-

ide production in grasshoppers, but notes that the higher rate was not maintained throughout the duration of exposure (eighty minutes).

This list is by no means complete but serves to show that increase in respiratory rate is a common result of the application of dilute solutions of many anesthetics to the most varied sorts of plant and animal tissues. The duration of the period of exposure to the anesthetics has been limited in most cases to twenty-four hours or less, and the possible effect of the increased respiratory activity on the weight of the tissue has not been intensively considered.

It is true that changes in weight do not always indicate changes in the mass of living substrate. Increase in weight by imbibition of water during early stages of embryos is a well-known phenomenon. Furthermore, Moore and Herdman ('14) showed that the live weight of a lobster actually increased during seven months' starvation. This is certainly not true of the weight during starvation in Planaria dorotocephala, as extensive work by Child ('19 a, and other papers) and Hyman ('19 b) has shown. The animals undergo progressive decrease in weight which is in general correlated with decrease in size, and imbibition of water is of minor importance as regards weight changes (see also Berninger, '11). The conditions, so far as weight is concerned, are essentially the same during the regeneration of pieces; as regeneration proceeds and the new individuals are reconstituted, the weight decreases (cf. Hyman, '19 c, pp. 70 to 75, col. 3). This decrease in weight in planaria during starvation or regeneration may be regarded as due, 1) to utilization of food in the alimentary tract during the early stages; 2) to utilization of reserves of materials in the tissues; 3) ultimately, to consumption of the protoplasm. The present paper presents data which are of interest in connection with the following questions: 1) Does the weight decrease more rapidly during the increase of respiratory rate induced by exposure to dilute anesthetics? 2) Are the conditions similar for several anesthetics? 3) If the exposure is continued, does the increased respiratory rate remain constant or does it continue to increase or tend to return toward the normal?

MATERIALS AND METHODS

The experiments reported here are of two general types, *a*) those dealing with the oxygen consumption and decrease in weight of pieces of Planaria dorotocephala regenerating in dilute solutions of anesthetics, and, *b*) those dealing with the oxygen consumption and decrease in weight of intact animals under similar conditions. Since the results as regards changes in oxygen consumption and weight were of the same nature in both, they will be considered together. The following anesthetics were employed: ethyl alcohol, chloroform, chloral hydrate, and, to a lesser extent, chloretone and ether. Particular attention was given to the purity of the substances; the best grades obtainable were secured; the alcohol was double or triple distilled. The strength of the solution of each substance used was determined by the viability of the animals. The maximum concentrations of these agents in which the animals will live for prolonged periods, varies with the physiological condition of the animals; young animals are more susceptible than old, and pieces greatly stimulated by section are more susceptible than pieces less stimulated. The solutions used were, therefore, concentrations in which the most susceptible animals or pieces lived without external evidence of injury. In general, with the exception of mol.1/10 alcohol, these concentrations were non-narcotizing. Intact animals were slightly more active in the anesthetic for a day or more, but after that period there was no appreciable difference in the behavior of the experimental animals and their controls. In mol.1/10 alcohol the animals were partially anesthetized for several days, but gradually recovered.

The intact animals, fifty or more, or the pieces immediately after section, were placed in a 500-cc. Erlenmeyer flask filled with the appropriate solution of the anesthetic. At the same time a like number of animals, or of pieces, as nearly as possible of the same size and physiological condition as the first set, were placed in another flask and the flask filled with water. This second set served as the control. The manner of cutting the pieces and of obtaining data on oxygen consumption has been

described in some detail elsewhere (Buchanan, '22 d) and need not be redescribed here. The oxygen analyses were made by the Winkler method with slight modifications of the method as employed by Birge and Juday ('11). The titrations were carried out in the presence of the dilute anesthetics; however, any possible error thus introduced is of no importance, since it would occur in both the blank sample and that drawn from the flask containing the animals.

After the oxygen analyses were made, the animals or pieces were weighed. Accuracy in weighing is obviously of primary importance; however, weighing living planaria with accuracy and without injury presents a number of difficulties. Drying must be avoided, since partial drying and handling in the semi-dry state produce injuries that disturb the normal respiratory conditions. On the other hand, wet weighing introduces the error of weighing varying quantities of water. It is admitted that these difficulties were only partially avoided. The most satisfactory method was by pouring the animals or pieces into a filter funnel lined with hard surfaced filter paper, and, after the liquid had drained off, drawing the paper over a clean glass plate until it adhered firmly. The animals or pieces were then picked up with a thin spatula and weighed in a Paar weighing tube. The weighing was done as quickly as possible and the animals promptly returned to the flasks. It is impossible by this method to avoid weighing small quantities of water that adhere to the animals, but the results obtained were consistent and apparently the error introduced was fairly constant; two sets of animals of as nearly as possible the same size and physiological condition show very slight differences in rates of oxygen consumption per milligram.

After the initial determination of the oxygen consumption per milligram per two hours was made, the flasks were set aside in darkness; that containing the control animals being filled with water and the one containing the experimental animals filled with the solution of the anesthetic. Both were tightly stoppered; a small air space was left under the stoppers to allow for changes in volume with temperature changes. The solution and water were replaced by fresh every forty-eight hours; in some experi-

ments, every twenty-four hours. Throughout the duration of an experiment at intervals of several days determinations of weight and amount of oxygen consumed per two hours per milligram were made, with temperature and other conditions the same as in the initial determination.

A word is necessary here concerning the factor introduced by motor activity of the animals during these tests of oxygen consumption. This source of error was reduced to the minimum by subjecting the flasks containing the animals to a temperature slightly lower than that of the room and keeping them in darkness during the test. Under such conditions the animals remain quiet and change position very little during the course of several hours. The animals and pieces subjected to the anesthetics were not more active than those in water except during the first day or two, and differences in motor activity therefore do not account for their differences in oxygen consumption. Motor activity may, however, account in part for certain minor irregularities appearing in the data of a single set of animals during the course of an experiment, and for slightly higher rate of oxygen consumption in the anesthetics during the first day (except in the case of alcohol, pp. 335 and 342).

DATA

With certain exceptions, the data are given in the form of curves, and for convenience in contrasting weight changes with metabolic changes the curves of the data on weight are superimposed on those of the data on oxygen consumption. In the oxygen consumption curves the ordinates represent fractions of a cubic centimeter of oxygen consumed per milligram per two hours, the abscissae representing the intervals between determinations. In the weight curves the ordinates represent the weight in milligrams and the abscissae are identical with those of the oxygen consumption curves. The examples given are selected as typical of the results in each case. No exceptions occurred and the only appreciable variations appeared to depend on the size of the animals, small animals attaining a higher rate of oxygen consumption than the controls earlier than large ani-

mals. The number of experiments on the effects of chloretone and ether is not sufficient to warrant publication of data that may be regarded as typical; the results thus far obtained are summarized briefly. A more complete study was made of the animals starving in alcohol than in the other agents; two sets were carried for ten weeks and two for six weeks. The susceptibility of the alcohol animals to lethal solutions of KNC at the end of six weeks was also tested.

Alcohol

Figure 1 gives the history of the oxygen consumption of a set of animals starving for six weeks in mol.1/10 alcohol, curve A, and of a control, curve B. Curves A1 and B1 are the respective curves of the changes in weight. In all, sixteen experiments were carried out with alcohol, the duration of exposure ranging from twenty-four hours (two experiments) to ten weeks (two experiments). Without exception the results agree, for the corresponding periods, with those represented in figure 1.[1]

Child ('15, and other papers) has shown that differences in rate of metabolism may be detected, under certain circumstances, by subjecting organisms to solutions of toxic substances, particularly KNC, in concentrations that are lethal, but so dilute that death changes require several hours before complete in all regions. In planaria the progress of death in such solutions can be easily followed. It has been found that the region in which the rate of metabolism is highest is the first to die, the death changes then successively involving other regions in order of rate. When the rates of death of two animals of different physiological condition are compared, the difference is a rough measure of their difference in metabolic rate. The reliability of this 'susceptibility method' as a rough measure of comparative rates of metabolism has been firmly established by comparison with direct quantitative

[1] It will be noted that the oxygen consumption of the control animals at the end of six weeks is much higher than at the beginning of the experiment. This increase of rate of metabolism in planaria after prolonged starvation has been denied by some investigators. The writer has found it occurring in every case in which the period of starvation was prolonged.

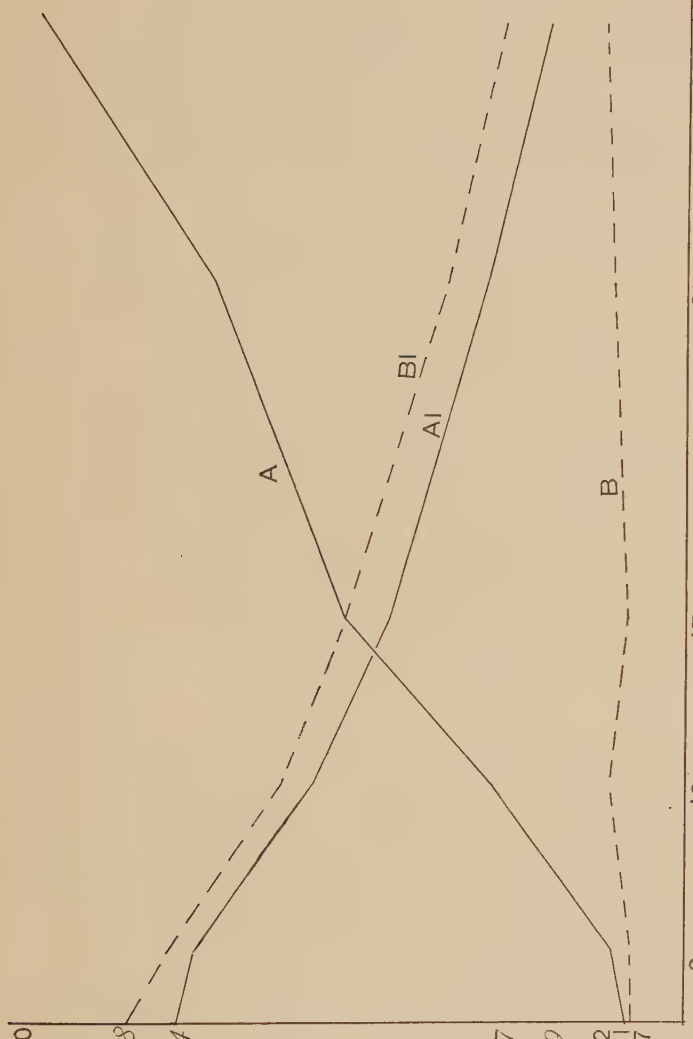

Fig. 1 A, oxygen consumption per two hours per mg. of 100 animals in mol.1/10 alcohol for six weeks. B, oxygen consumption of control. A1, weight of alcohol animals; B1, weight of control. Animals 14 to 16 mm. in length and fed 72 hours before start of experiment. All tests conducted at 20½°.

measurements of carbon dioxide production and oxygen consumption, by comparison with the progress of death due to lack of oxygen, and with other data.[2]

The susceptibility at the end of six weeks of four sets of ten each of the animals whose histories are given in curve A and curve A1 in figure 1 was tested and compared with that of the controls. The exact procedure in each test was as follows: Twenty animals of as nearly as possible the same size were selected with great care, ten from the alcohol stock and ten from the control, and placed in small Erlenmeyer flasks, the alcohol animals in one and the controls in another. The flasks were then filled with a fresh solution of KNC, mol.1/1000, and both flasks tightly stoppered. The flasks were then set aside under as nearly as possible the same conditions as regards light and temperature and at regular intervals the animals were examined with the aid of a hand lens and the progress of death in each noted. For convenience, Child has designated five stages in the progress of death and disintegration in planaria. In the present work, Child's criteria were followed and the number of animals in each stage was tabulated separately for each flask at every examination. Figure 2 is a graphic presentation of the results of all experiments, curves A, 1, 2, 3, and 4, being based on the data of the alcohol animals, and curves B, 1 and 2, on the data of the controls. In plotting curves of this sort, it is necessary to assign a numerical value to the proportion of the whole animal remaining alive at each stage of disintegration. Thus stage I is assigned the value 10, and since all the animals are in stage I at the start of the experiment, the value for each flask is 100 (plotted as ordinates). Stage II is assigned the value 8, and the combined value of the control animals at the end of two hours is reduced from 100 by the passing of some of the animals into stage II. In the alcohol animals the reduction from 100 is much greater, since more of the

[2] The discrepancy between Lund's results ('21 a) on the effect of *KNC* on the rate of oxygen consumption of Paramecium and those of numerous investigators on the inhibitory effect of the cyanides on oxidations does not detract from the usefulness of the susceptibility method. On the contrary, it would seem that the apparent exception in the case of Paramecium demands further analysis.

animals have passed into stage II, and some even to stage III. The determinations for succeeding hours are plotted in the same manner. This method of plotting susceptibility curves is slightly modified from Child's method.

The striking difference between the curves of the alcohol animals and those of the controls requires no extended comment. Lack of material prevented further experiments of this sort, but

Fig. 2 Susceptibility of animals from figure 1, to mol.1/1000 *KNC*. A, 1, 2, 3, and 4, animals from alcohol set; B, 1 and 2 control animals.

in view of the very definite and almost identical results obtained in the four experiments tried, it is believed that further evidence of differences in susceptibility is not necessary. The results receive support in some work of Hunt ('07) who found that mice accustomed to alcohol feeding succumbed to methyl cyanide much more quickly than did control animals.

Two sets of larger animals with proper controls were carried for ten weeks with essentially the same results as in the examples given in figure 1, except that with the larger animals the increase in amount of oxygen consumed per two hours in the alcohol animals was not so rapid as in curve A, figure 1, the high point not being attained until the tenth week.

Figure 3 gives the record of a set of pieces regenerating in mol.1 10 alcohol for fourteen days, and of the control. It will be noted that the scale of the abscissae differs slightly from that in figure 1; however, the curves in figure 1 and figure 3 show the similarity in the results of the two experiments.

It has been found that when pieces are isolated from the body of planaria, a period of stimulation ensues which persists for twelve to twenty-four hours. This stimulation is marked by a distinct increase in oxygen consumption and carbon dioxide production of the pieces, by increased susceptibility to poisons, and by other evidences of increased metabolic rate (Child, '14, and other papers; Hyman, '19 c; Robbins and Child, '20). Indirect evidence indicates that in all probability this stimulation is of nervous origin except in the cells at and near the cut surfaces of the piece (Buchanan, '23). Strong solutions of chloroform, ether, chloral hydrate, and chloretone inhibit this stimulation and thus to an appreciable degree prevent the increase in oxygen consumption that normally follows section. It has been found possible by this method to control the regeneration of new individuals to some extent. The nature of the conditions controlling regeneration and their experimental control has been discussed elsewhere and will not be entered into here. From the results obtained in regeneration experiments and from the behavior of the animals there is clear evidence that mol.1 10 alcohol narcotizes the nervous and other transmission paths; however, the oxygen consumption of the pieces in this concentration of alcohol is higher immediately after section than in control pieces. The explanation offered for this difference between the effect of alcohol and that of strong solutions of other anesthetics is that the oxygen consumed is concerned in oxidizing the alcohol and that this source of oxygen absorption masks any inhibitory effect of the agent on the stimulation of

section (Buchanan, '22 d). As will be noted later (p. 349), this explanation seems well founded. It seems probable, therefore, that in the results given in figure 1 and figure 3 the oxygen consumption is general in the tissues and does not result from a purely nervous effect. The same is also probably true of the effects of the other anesthetics when employed in non-narcotizing concentrations.

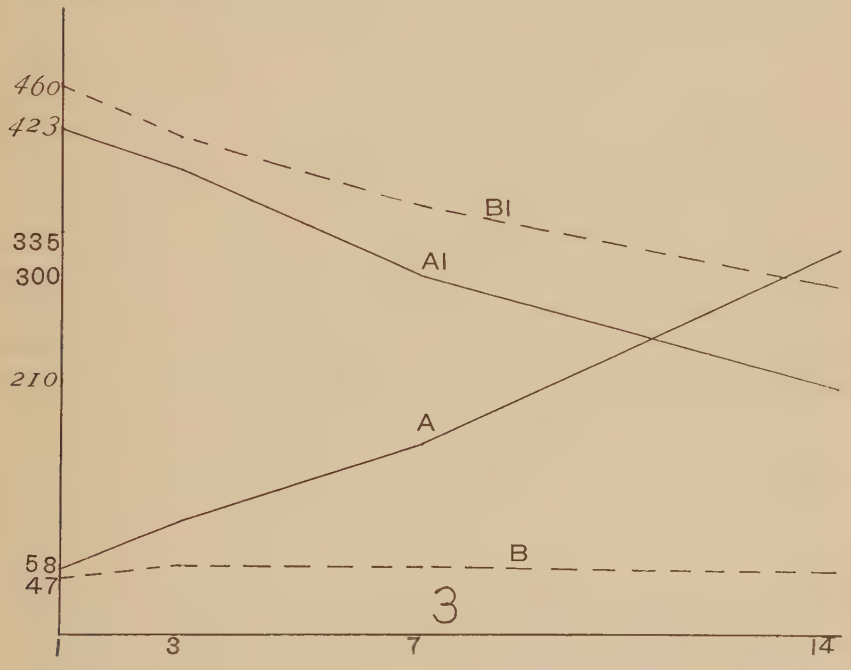

Fig. 3 A, oxygen consumption per two hours per mg. of pieces of Planaria regenerating in mol.1/10 alcohol for two weeks; B, oxygen consumption of control pieces. A1, weight of pieces in alcohol; B1, weight of control pieces. All tests at 20½°.

In figure 1 the curves A1 and B1 show that the weight of the animals decreases at practically the same rate in both alcohol and control sets. This is somewhat unexpected in the light of the fact that the oxygen consumption of the alcohol animals mounts above that of the control steadily and rapidly throughout the duration of the experiment, and also in view of the fact

that the greatly increased susceptibility to KNC suggests that the intrinsic metabolic processes involving oxidations are greatly stimulated. In figure 3 the weight of the pieces in alcohol decreases slightly more rapidly than that of the control. The results in other experiments are intermediate between these two examples or else like figure 1. The data justify the conclusion that the decrease in weight in alcohol when compared with controls is not related intimately to the increase in rate of oxygen consumption.

Chloroform

Figure 4 (plotted on the same scale as fig. 3) shows the history of the oxygen consumption of a set of pieces regenerating in mol. 1/3000 chloroform for eleven days, curve A, and of the control, curve B. Curves A1 and B1 are their respective weight curves. Comparison of figure 4 with the alcohol results in figure 1 and figure 3 shows that while at the end of eleven days the animals in the chloroform consume slightly more oxygen than their controls, the differences are much less than the differences between the alcohol animals and their controls at the corresponding period. It must be pointed out, however, that the data are not strictly comparable, for the solutions are not iso-molar. A study of iso-molar solutions of these agents is not practicable with planaria; the solubility of chloroform in water is so slight that it is not possible to make a mol.1/10 solution. Furthermore, even a mol.1/1000 solution of chloroform is so toxic that the animals are killed in less than forty-eight hours. On the other hand, mol.1/3000 alcohol (ca. 1/600 per cent) is so dilute that its effects cannot be detected with certainty.

Comparison of the weight curves of alcohol and chloroform shows that the decrease in chloroform in eleven days is much greater when compared with the control than in the case of the animals subjected to alcohol. This was very generally true in the experiments with chloroform, although the decrease in weight was not always as great as in the example given.

The work with chloroform includes fourteen experiments, eight with pieces and six with intact animals, the periods of

exposure ranging from three days to two weeks. In all experiments the results agree in substance with the example given in figure 4.

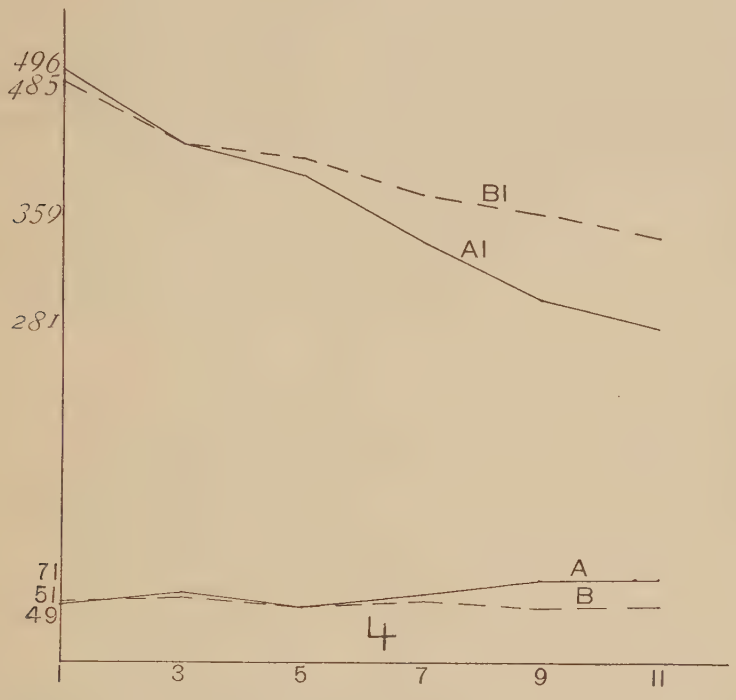

Fig. 4 A, oxygen consumption per two hours per mg. of pieces of Planaria dorotocephala regenerating in mol.1/3000 chloroform for 11 days; B, oxygen consumption of control. A1, weight of pieces in the chloroform; B1, weight of control. All tests at 21½°.

Chloral hydrate

Figure 5 shows the oxygen consumption results obtained in two weeks exposure of animals to mol.1/825 chloral hydrate, curve A, and the control, curve B, the weight curves being designated as A1 and B1, respectively. The data show that the increase in oxygen consumption in this concentration of chloral hydrate is greater than in M/3000 chloroform, but much less than in M/10 alcohol. The rate of oxygen consumption does not

begin to rise at once, as is the case in alcohol. For several days it remains the same or even slightly below that of the controls. Some time after the fourth day, however, the rate of oxygen consumption of the animals in chloral hydrate rises above that of the controls and continues to rise throughout the duration of the experiment. As regards decreases in weight, in most of the fourteen experiments with chloral hydrate the decrease in weight during the early periods of exposure was slightly less than that of the controls. Approximately coincident with the rise of the rate of

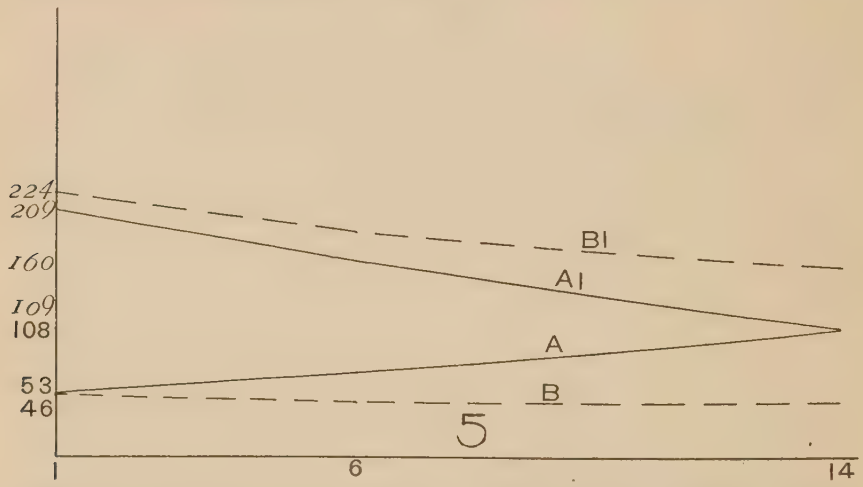

Fig. 5 A, oxygen consumption per two hours per mg. of 75 animals starving in mol.1/825 chloral hydrate for two weeks; B, oxygen consumption of control. A1, weight of animals in chloral hydrate; B1, weight of control. Animals fed 72 hours before start of experiment. All tests conducted at $18\frac{1}{2}°$.

oxygen consumption above that of the controls (fourth to sixth day), the weight begins to decrease more rapidly so that at the end of ten to fourteen days the total decrease of the experimental animals was distinctly greater than that of the controls. The possibility of loss of substance due to disintegration is not excluded in these results. Highly susceptible animals, i.e., young animals or animals subjected to prolonged starvation, show some disintegration of the tissues of the head if the exposure to M/825 chloral hydrate is continued beyond ten days. This effect is more pronounced and appears sooner when the room temperature is high.

The disintegration is regarded as external evidence of a lethal effect which may be concerned to some extent in weight decreases in experiments in which there is no external evidence of disintegration. The weight decrease in chloroform probably also includes some slight loss of disintegration. Six experiments with pieces and eight with whole animals starving in M/825 chloral hydrate were carried out, the periods of exposure ranging from five days to two weeks. The results agree in general with the example given in figure 5. Weight changes are more distinct in the experiments with whole animals and the results necessitate the modification of a statement in an earlier paper (Buchanan, '22 d) that the weight decreases at approximately the same rate in both experimental and control series.

Chloretone

Six experiments with pieces regenerating in M/1000 chloretone for two weeks were performed. The study is by no means complete, but the results obtained may be briefly summarized: The oxygen consumption of the pieces in the chloretone rises above that of the control, the rise continuing from the third day to the fourteenth. However, in no case was the oxygen consumption at the end of the fourteenth day more than one and one-half times that of the control. The weight of the animals in the chloretone decreases more rapidly than that of the controls during the later periods of exposure.

Ether

Six experiments with pieces regenerating in M/300 ether for two weeks were performed. Although the processes of regeneration are greatly affected by this concentration, and stronger solutions are more or less lethal, the oxygen consumption of the pieces in the ether did not vary appreciably from that of the controls at any time, nor did the weight decrease more rapidly. These results are not regarded as evidence that ether may not produce effects similar to those of the other agents employed. The explanation of the non-occurrence of the increase in oxygen con-

sumption and of the lack of effect on weight is probably to be found in the concentration used; results similar to those obtained with the other agents might be expected in other concentrations of ether.

GENERAL CONSIDERATIONS

In view of the fact that the results given here are in some respects different from those obtained by others, a further word concerning the possibility of error seems advisable. A number of possible sources of error other than those discussed above (page 336) remain to be considered.

The striking increase in oxygen consumption of the animals starving in these solutions, especially in alcohol, may be due to the accumulation of the micro-organisms in the flasks and not to the respiration of the animals. The data on susceptibility reduce this possibility. Furthermore, the flasks were carefully washed out at each determination of oxygen consumption, while the animals were in the filter funnel during the process of weighing. At New Haven, and frequently at Chicago also, it was necessary to heat the water before using because of its supersaturation with oxygen. It was noted that heating the water and then cooling and aerating by shaking thoroughly made no difference in the results of the tests.

A second possibility is that the animals give off during exposure to anesthetics some iodine-absorbing substance, the output of which increases from day to day and which by uniting with free iodine renders the titration of the samples inaccurate and worthless. This possible source of error is rendered exceedingly remote by the following test. If some of the liquid is withdrawn from the flask after the sample has been taken, and shaken with air, its iodine-absorbing power is found to be reduced approximately to that of the stock solution when saturated with oxygen at the same temperature.

Verźar ('12) gives data showing that the oxygen consumption of certain tissues is to an appreciable extent dependent on the oxygen tension in the environment, while others, notably the salivary gland, are more completely independent of the oxygen

concentration. In the case of the kidney Verźar shows that low oxygen tension increases oxygen consumption. Lund ('21 b) has presented data which indicate the minimum concentration of oxygen necessary to maintain the normal rate of oxygen consumption of Planaria agilis. The possibility of a relation between oxygen concentration below a certain minimum, and oxygen consumption has been recognized in this and previous work, and every precaution taken to assure an adequate supply in the flasks at all times.

From the data and from consideration of possible sources of error it is reasonable to conclude that the increase of oxygen consumption of planaria in the presence of dilute anesthetics is continuous and cumulative throughout extended exposure. There appear to be but two possible factors involved: either the oxygen is utilized in oxidizing the substance or the normal respiratory metabolism of the protoplasm is increased, or both factors may be concerned. In the case of alcohol, the facts indicate that the major part of the excess oxygen absorbed is concerned in oxidation of the alcohol. Ethyl alcohol is easily oxidized and Pringsheim ('08) and others have shown that it is oxidized readily and rapidly in organisms. Cushny ('12) states that in the human body at least 95 per cent of the alcohol taken in is disposed of in this way.

The lack of correlation between the very striking increase in oxygen consumption of planaria and weight changes apparently indicates that the alcohol is oxidized by some mechanism which is not closely related to the intrinsic anabolic and catabolic reactions in the protoplasm. This would not accord with the conclusions of a number of investigators, that the oxidation of alcohol is substituted for the oxidation of carbohydrates and that the presence of alcohol thus tends to the accumulation or retention of these substances. Under such conditions, one would expect to find the weight of the alcohol animals decreasing less rapidly than that of the controls. The discrepancy may be only apparent, however. The very greatly increased susceptibility to KNC strongly indicates that the intrinsic oxidative metabolism is greatly stimulated. One would expect this cumulating increase

in oxidations to be more or less closely accompanied by an increasing requirement for oxidizable substances. Under such conditions, weight changes might not show any retention or accumulation of carbohydrates.

As regards the results with chloroform, it is probable that the relatively slight increase in oxygen consumption is largely due to stimulation of the normal respiratory exchange and that the oxygen absorbed is very little concerned in oxidizing the chloroform, since this substance is not readily broken down in organisms.[3] This probability is supported by the fact that the weight of the animals decreases with relative rapidity in chloroform. That it is possible for small quantities of certain substances to induce increases in oxygen consumption was shown by Warburg ('10), who found that dilute solutions of sodium hydroxide doubled the oxygen consumption of sea-urchin eggs even without entering. Warburg also found that ammonium hydroxide, which penetrated the cells, also produced slight increases in oxygen consumption when employed in dilute solutions.

The data on the effects of chloral hydrate indicate that this substance is oxidized to some extent by the organism, although the data are not as suggestive as in the case of alcohol. The decrease in weight in chloral hydrate indicates that the decreases in weight in alcohol and chloroform, particularly the latter, are not necessarily due to continuous dissolving out of lipoids and fats, since chloral hydrate, which has similar effects on weight, is only slightly soluble in these substances. The chloral hydrate data also show that an increase in oxygen consumption occurs in both water and lipoid soluble anesthetics when employed in dilute concentrations.

No examination was made of the waste products and carbon dioxide output. Undoubtedly such analyses would indicate more clearly the nature of the processes of metabolism affected by weak solutions of anesthetics. It may be added that close inspection

[3] Gwathmey ('18, p. 309) cites work by Thien and Fischer showing that chloroform may remain for twelve days after administration in the human body, a small fraction being broken down and the remainder excreted with expired air and in the urine.

of the animals in which the oxygen consumption was abnormally high—for instance, during the later period of extended exposure to mol.1/10 alcohol—failed to reveal any evidence of imbibition or of shrinkage.

SUMMARY

When individuals or pieces of Planaria dorotocephala are subjected continuously to mol.1/10 alcohol, the oxygen consumption of the animals increases much more rapidly than that of control animals from day to day. With animals of moderate size the oxygen consumption in alcohol at the end of six weeks is 700 per cent that of control animals in water. The susceptibility of the animals to lethal solutions of KNC also increases markedly.

The weight of the animals in the alcohol decreases slightly more rapidly than that of control animals (ca. 25 per cent).

Subjecting animals to mol.1/3000 chloroform for two weeks induces a slight increase in oxygen consumption. The weight of the animals decreases more rapidly than that of control animals (ca. 75 per cent).

Subjecting the animals to mol.1/825 chloral hydrate induces a rate of oxygen consumption that is distinctly higher than that of control animals. The weight of the animals in chloral hydrate decreases more rapidly than that of the control (ca. 40 per cent).

Incomplete work indicates that exposure to mol.1/1000 chloretone also induces an increase in oxygen consumption above that of control animals and also that their weight decreases more rapidly. Similar experiments with mol.1/300 ether failed to show any effect of this concentration on oxygen consumption and decrease in weight.

The probability of oxidation of these agents by the organism is discussed, together with the probability of stimulation of other oxidative processes in the presence of these agents.

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16 (2248)

The oxygen consumption of thyroid and diiodotyrosine-fed tadpoles.

By O. M. HELFF (by invitation).

[*From the Osborn Zoological Laboratory, Yale University, New Haven, Conn.*]

It has been known for some years that thyroid-fed tadpoles undergo precocious metamorphosis, and more recently the fact has been demonstrated that diiodotyrosine has a similar effect upon amphibian larvæ. Students of amphibian metamorphosis have assumed that an increase in metabolic rate occurs during transformation, and the statement is even made that metamorphosis is due to increased metabolism. So far as the writer is aware, however, no attempt has been made to test the validity of these assumptions. It seemed worth while, therefore, to measure the metabolic rate of precociously metamorphosing tadpoles at various stages of transformation. Since oxygen consumption is considered a rough gauge of metabolic rate, this method was employed.

EXPERIMENTAL

During the months of June, July, August and September, 1923, two hundred *Rana pipiens* tadpoles of approximately the same size and developmental stage were collected. They were fed for two days on Spirogyra and then starved for an equal period, preparatory to running the initial test for oxygen consumption. Dessicated thyroid, containing .20 per cent of iodine, and diiodotyrosine were the agents employed to accelerate metamorphosis. The animals were placed in small jars of water, one tadpole to a container, and kept at a constant temperature of

22 ± 1° C. It was found that continued feedings of thyroid lowered the resistance of the animals to such an extent that death occurred before advanced stages of metamorphosis were reached. Consequently, the method employed was to feed the tadpoles one or two days with thyroid and then keep the animals in solutions of diiodotyrosine which, although it greatly hastens metamorphosis, does not weaken the larvæ to the extent that is true of continuous administrations of thyroid.

Successive stages of metamorphosis were arbitrarily selected: the normal tadpole showing no indications of transformation was designated stage one, the metamorphosed animal whose left fore leg had appeared and been free for at least a day was designated stage 8. The remaining six stages represented intermediate points of development. On the average, a thyroid-diiodotyrosine-fed tadpole passed from stage 1 to stage 8 in a period varying from eight to eleven days. Tadpoles so metamorphosed and whose oxygen consumption had been measured several times, invariably died after reaching stage 8, and consequently, no measurements were made on animals with both fore legs through the operculum.

The Winkler method was used throughout in determining the oxygen consumption, the water used in the experimental flasks being in all cases maintained at 24° C. It was found necessary to etherize tadpoles of stage 1 (normal animals) during the test in order to eliminate errors arising from the activity of the individuals under operation. Etherization was found unnecessary in all succeeding stages since tadpoles undergoing transformation remain quiescent for considerable periods. All individuals were weighed accurately to the milligram, whenever tested, in order to determine the oxygen consumption per gram weight.

RESULTS

The results obtained show, beyond doubt, that there is a progressive increase in oxygen consumption per gram weight of metamorphosing tadpoles. Between stages 1 and 2 there is an increase of 48 per cent. Stages 3 and 4 show a still further increase of 59 and 76 per cent respectively. The next two stages (5 and 6) show a somewhat lessened increase over stage 1 as compared with stage 4 or 61 and 54 per cent respectively. The last two stages (7 and 8) give a respective increase of 71 and 79 per cent over the normal or stage 1 consumption, stage 8, as

was expected owing to the advanced stage of metamorphosis, possessing the greatest increase per gram weight of any stage tested. Table I gives the average actual amount of oxygen in cubic centimeters consumed per gram weight in one hour for the eight stages, while Fig. 1 represents graphically the per cent increase in oxygen consumption of the same individuals.

TABLE I.

Average Oxygen Consumption in cc. per gm. wt.

No. Tested	Stage 1	2	3	4	5	6	7	8	Av. Increase
32	.157	.233							.076
19	.160		.254						.094
33	.160			.282					.122
36	.178				.286				.108
16	.171					.264			.093
27	.174						.298		.124
10	.192							.344	.152

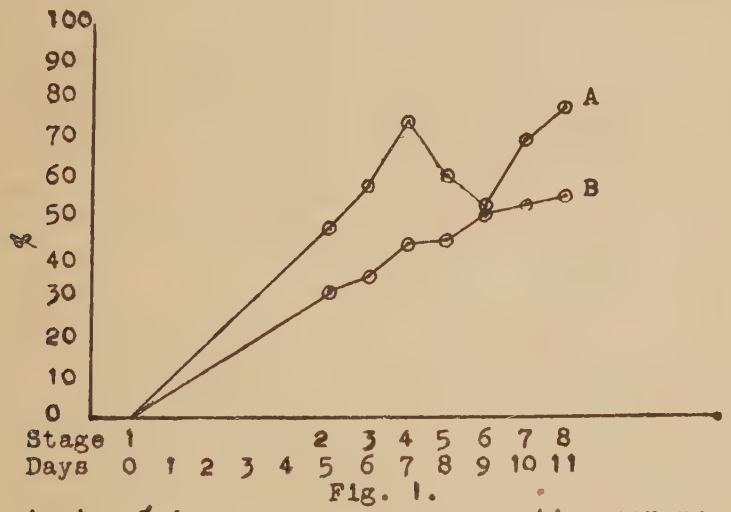

Fig. 1.

A. Av. % increase oxygen consumption per gm. wt.
B. Av. % decrease in total weight.

By referring to Table II, it is at once evident that there is a progressive decrease in total weight as the tadpole attains each successive stage of metamorphosis. Fig. 1 emphasizes this fact by graphically representing the per cent decrease in total weight as compared with the normal or stage 1 weights of the same individuals. If the per cent increase of oxygen consumption per

gram weight was of the same percentage as the per cent decrease in total weight at any given stage, the conclusion would be that no increase in total oxygen consumption had occurred. In reality, however, the data show a progressive increase in total oxygen consumption with an increasing stage of metamorphosis.

TABLE II.
Average Weight in gms.

No. Tested	Stage								Av. Decrease
	1	2	3	4	5	6	7	8	
32	1.394	.942							.452
19	1.451		.922						.529
33	1.409			.793					.616
36	1.398				.765				.633
16	1.375					.662			.713
27	1.400						.643		.757
10	1.415							.605	.810

The bulk of the increase per gram weight, however, is accounted for by the large decrease in total weight. The falling off of the per cent increase in oxygen consumption in stages 5 and 6 is in a large measure due to the fact that many of the tadpoles tested at this stage were in a weakened condition and died within five to ten hours after the test and before reaching stage 6 or 7. In other words, the critical period in thyroid-diiodotyrosine metamorphosis seems to be during these stages. Once past these stages, the tadpoles recovered their vigor and hence the increased oxygen consumption in stages 7 and 8.

The effect of etherization showed that, if anything, anesthetization by this agent increased oxygen consumption. In fact, the average increase per gram weight of sixteen individuals so anesthetized, amounted to 19 per cent, which also represents the per cent increase of total oxygen consumed, since the normal and etherized tests were run within an hour of each other during which period no reduction in weight had occurred. This fact, then, adds emphasis to the percentage increases of oxygen consumption recorded in the later stages.

DISCUSSION

The writer was fortunate in obtaining a full series of tadpoles of the same species used in the oxygen tests, which were undergoing normal metamorphosis. Several striking dissimilari-

ties were noticeable when compared to a similar series of thyroid-diiodotyrosin metamorphosed tadpoles. The greatest difference was found in the atrophy of the tail as compared to the growth of the limbs. For example: a normally metamorphosing tadpole, which had succeeded in protruding the left fore limb through the operculum, possessed a tail in the same stage of atrophy as was to be found on a typical stage 4 thyroid-diiodoytrosine metamorphosed tadpole, where the left fore limb would require several days yet to appear. In other words, the fore limb appeared much earlier in relation to the atrophy of the tail in the normal series than in the thyroid-diiodotyrosine series.

Other such differences in correlation were observed, making it clear that the thyroid-diiodotyrosine type of metamorphosis did not permit the atrophy, autolysis or growth of certain parts to proceed in the normal manner. This undoubtedly accounts for the high mortality rate met with in all series of animals so metamorphosed. It would also account, in a large measure, for the considerable variation in oxygen consumption of individuals of the same metamorphic stage. This variation in oxygen consumption made it necessary to base the consumption at any given stage on the average of a considerable number of animals.

CONCLUSIONS

1. *Rana pipiens* tadpoles, metamorphosed with dessicated thyroid-diiodotyrosine exhibit a progressive rise in metabolic rate as evidenced by oxygen consumption per gram weight. In stage 8 the metabolic rate reaches a maximum increase over that of stage 1 of approximately 79 per cent.

2. Precociously metamorphosed tadpoles increase their total oxygen consumption regardless of decreasing weight.

3. Thyroid-diiodotyrosine-fed tadpoles undergo a gradual decrease in weight amounting to 57 per cent in stage 8.

4. Etherization of stage 1 tadpoles (normal animals) results in approximately 19 per cent increased oxygen consumption.

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IODINE AND AMPHIBIAN METAMORPHOSIS.[1]

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Recently the claim has been made that the metamorphosis of urodele larvæ such as axolotl and amblystoma differs from that of anurans in that transformation in the caudate amphibia is independent of iodine and subject only to the influence of the thyroid gland. Experiments are described in this paper which invalidate this conclusion and indeed, render it impossible to understand such a view. The rôle of iodine in metamorphosis is also discussed, and evidence presented showing that the physiologic response (metabolic changes) of mammals to thyroid administration and the metamorphic changes of amphibian larvæ following iodine treatment are probably due to quite different causes and hence not to be compared.

Acknowledgement is due Mr. William Anderson for his labor in iodizing the various compounds employed.

The axolotls came from New Mexico, and were obtained through the courtesy of Mr. J. N. Gladding of Albuquerque.

I. Experiments on Thyroidectomized Axolotls.

The thyroid glands of eight animals were extirpated. One larva was extremely large, measuring over a foot in length. The remaining larvæ averaged seven inches total length. Few technical difficulties are involved in removing the thyroid glands from animals as large as the axolotl. The larvæ were anesthetized in chloretone solutions, placed upon their back under a low-power binocular microscope, and the hyobranchial region strongly illuminated. A median incision was made through the skin and superficial muscles, extending from the posterior edge

[1] Part of the expense of this investigation was defrayed by a grant from the Elizabeth Thompson Science Fund.

of the hyobranchial region to the symphysis of the mandible. The skin and superficial muscles were pulled apart on either side and pinned down, exposing the geniohyoid and ceratohyoid muscles. The thyroid glands are located in the triangle on each side of the median line, formed by the geniohyoid and ceratohyoid muscles. The large size of the glands and their intimate relation to the blood vessels which traverse the triangle render their location easy. They can be removed from both sides of the animal with but little injury to the blood vessels if first dissected free with a razor-edge needle and gently pulled out with a fine-pointed pair of forceps. Even if the blood-vessels are injured and considerable bleeding occurs, the post-operative effects upon the animal are negligible. It is surprising how much surgical manipulation an axolotl can withstand without showing any post-operative symptoms. Following gland removal the skin and superficial muscles are sutured along the median line. The animals quickly recover from the anesthetic and appear to suffer no ill effects from the operation. Food was witheld for four or five days after thyroidectomy in order to prevent any possibility of tearing the sutures while swallowing.

The thyroidless animals were kept for five months in large concrete tanks through which fresh water ran constantly. The food consisted of worms, insect larvæ, and occasionally, pieces of fresh liver.

Five months following thyroid removal, three animals were injected twice, at five day intervals with eighty milligrams of tyrosine in which two atoms of iodine had been substituted for two hydrogen atoms of the molecule, forming the well-known compound 3–5–diiodotyrosine. The animals metamorphosed within seventeen days following the first injection.

Three control thyroidless axolotls injected with equal quantities of pure tyrosine and 3–5 dibromtyrosine, *i.e.*, tyrosine in which two bromine atoms had been substituted for two hydrogens, failed to transform. Later, one of the controls was injected with a third dose of eighty milligrams of dibromtyrosine but with negative results. Dissection of the metamorphosed iodotyrosine-injected animals showed no trace of thyroid tissue present.

Rogoff and Marine ('17) showed that iodized blood serum accelerates metamorphosis of normal tadpoles, and that the

globulin fraction of the serum contained most of the iodine. It was decided to try injecting iodoserumglobulin into thyroidless axolotls, controlling the experiment by injections of equal amounts of non-iodized globulin. The blood of beef was used.

Three axolotls were each injected twice, at eight-day intervals with 110 milligrams of iodoserumglobulin. Metamorphosis resulted within twenty days following the first injection. One of the axolotls had previously served as a control in the iodotyrosine experiment and had been injected with pure tyrosine. The results were negative and as the larva showed no indications of metamorphosis several weeks later it was utilized in the iodoserumglobulin experiment. Two control thyroidless animals (previously used as controls in the iodotyrosine work) injected with large amounts of non-iodized serumglobulin failed to transform.

Dissection of the metamorphosed iodoserumglobulin-injected animals showed two of them to have no vestige of thyroid tissue, but the remaining one had a portion of the gland present on the left side, an amount about equal to a third of the entire thyroid. One of the controls was then injected with iodoserumglobulin and metamorphosed twenty-one days after the first injection; dissection of this animal also showed a small nodule of thyroid tissue present. Thus four axolotls were metamorphosed by injections of iodized serumglobulin; two of the animals were completely thyroidectomized, and two only partially. No very marked difference in the time or rate of metamorphosis was noted between the partially and completely thyroidectomized animals. The axolotls possessing remnants of glands were the first to show signs of transformation following injection, but the time difference was not over three days in any case. The fact that both types of animals metamorphosed within a few days of each other may have been due to the large amount of iodoserumglobulin injected. It is probable that partially thyroidectomized animals will be found to respond by metamorphosis to considerably smaller doses than thyroidless animals. Axolotls were not available to test this point.

Considerable difficulty was experienced injecting the globulin intraperitoneally. The solution of the problem while crude was effective. A small incision was made through the ventral body wall sufficiently large to admit the end of a graduated pipette

and the globulin was injected in powdered form. This method because of the ease with which it could be performed was also utilized in the tyrosine experiments. Forced feeding of the animals with the various substances by means of a pipette thrust down the œsophagus into the stomach, might have been as effective as injections, but the animals usually regurgitate some of the material, at any rate this was found to be the case in an earlier experiment where desiccated thyroid tissue was administered by forced feeding.

The pigment pattern characteristic of adult Amblystoma tigrinum is not completely developed in axolotls until several weeks following metamorphosis.

Jensen ('21) metamorphosed axolotls (with intact thyroid apparatus) by injections of iodized casein, iodoserumglobulin, and iodoserumalbumin. He also removed the thyroid glands of axolotls and attempted to metamorphose the larvæ by injections of iodized proteins but his animals died. He concluded that such iodized proteins are highly toxic for thyroidless animals but not for those possessing normal glands. In my own experiments the axolotls withstood the injections of iodized amino-acid and serumglobulin as well as those with pieces of the gland present. There was nothing in the behavior of the animals to indicate that iodized substances are more toxic for thyroidless axolotls than for normal animals. Iodized substances are certainly not more toxic for thyroidless anuran tadpoles than for normal larvæ.

In an earlier experiment than those recorded here three axolotls were thyroidectomized and injected with large doses of iodotyrosine. The animals were kept in ordinary glass aquaria without running water. All of the animals died within ten days following injection. In later experiments the injected axolotls were kept in large concrete tanks, filled to capacity and with fresh water running constantly. None of the animals died. Intraperitoneal injections of large doses of either iodotyrosine or iodoserumglobulin has a marked depressing effect upon thyroidless axolotls; the animals are sluggish, move about very little, refuse food, and show indications of weakness for several days or even a week following injection.[2]

[2] A large axolotl was thyroidectomized and kept for eight months then twice injected at eight day intervals with large amounts of iodized casein. Metamorphosis resulted within twenty-nine days.

The experiment shows beyond doubt that thyroidless urodele larvæ respond to iodized substances in precisely the same manner as thyroidless and pituitaryless anurans. The importance of iodine in the amino-acid and protein molecule in order to render these substances effective in inducing metamorphosis is clearly demonstrated.

II. Experiments on Larval *Spelerpes bislineatus*.

H. H. Wilder years ago ('99) called attention to *Spelerpes* as a favorable object for experimentation. Unlike most urodeles, the larvæ of *Spelerpes* can be obtained at any season of the year and are easily kept under laboratory conditions. The larval life of this form has not been adequately investigated, consequently little is known concerning it. I. W. Wilder ('22) has been engaged for some years in studying the relation of growth to metamorphosis but to date has published only a very brief summary of the results. The data indicate a large range of variation in the size and age of the animals at transformation, with an average larval life of two years.

Eighty animals were used in the experiment, varying in size from 23 mm. to 53 mm. total length. They were separated into four groups of twenty animals each, equal numbers of large and small larvæ being represented. One group of twenty animals was kept in a large aquarium and given plenty of food. The three remaining cultures were subdivided into smaller lots of ten animals each and kept in glass containers in 250 cc. of tap water water through which compressed air bubbled constantly. *Spelerpes* larvæ soon die if kept in small amounts of water unless it is kept cool and well ærated. Twenty individuals were reared in tyrosine solutions representing 120 mg. per 250 cc. of water; another twenty were kept in equivalent solutions of 3-5 dibromtyrosine; twenty more in equal concentrations of 3-5 diiodotyrosine. The animals were fed sparingly during the experiment. Once each week they were removed from the tyrosine solutions and placed in large containers holding 6,000 cc. of water, plentifully supplied with food and allowed to feed for thirty-six hours after which they were returned to the solutions.

The experiment began October 17. Thirteen days from the date the animals were placed in the solutions, two large larvæ of

the iodotyrosine culture showed marked gill and tail-fin reduction. None of the other animals of this or other cultures showed any change. However, by November 5, *i.e.*, twenty days from the first administration of iodotyrosine, all of the larvæ kept in solutions of this substance had metamorphosed. The external gills had disappeared, the gill clefts had closed and the tail fin was completely resorbed. The animals left the water and crawled up the sides of the container.

Examination of the tyrosine and dibromtyrosine cultures showed no indications of metamorphosis. The animals of these cultures were kept in the solutions for a month longer but metamorphosis was not induced. At the close of the experiment exceedingly strong concentrations of tyrosine and dibromtyrosine were employed but with negative results. The larvæ of the normal culture kept in the large aquarium and plentifully supplied with food likewise failed to metamorphose or to show any indications of transformation a month after the animals of the iodotyrosine culture had completed the process.

The results of this experiment are quite clean cut and admit of but one interpretation: It is the iodine within the tyrosine molecule that is responsible for the induced metamorphosis because the tyrosine and dibromtyrosine were ineffective either in weak or strong concentrations when administered over comparatively long periods.

Efforts were made to thyroidectomize the larvæ but without success owing to the extremely small size of the thyroid glands. The thyroid apparatus of *Spelerpes* larvæ of 47 mm. total length does not contain enough of the physiologically active hormone to induce metamorphosis in anuran tadpoles when heteroplastically transplanted. This experiment was attempted by Mr. O. M. Helff of this laboratory but without success.

Judging by the positive results obtained with thyroidless axolotls, it is highly probable that thyroidless *Spelerpes* would react by rapid metamorphosis if injected with, or reared in solutions of iodotyrosine. It is interesting to note that both large and small larvæ metamorphosed within twenty days, though the differences in size were great, varying as they did from 23 mm. to 52 mm. total length. Probably some of the smaller animals were considerably younger than the larger ones, however, it is

impossible to make any definite statements on the point until more is known about the relation of size to age in the larvæ of this urodele.

The fact is well known that the thyroid gland of vertebrates exhibits a remarkable selective action in regard to iodine absorption, taking this element from the blood and synthesizing it into the thyroid hormone by the addition of other substances. In view of this property of thyroid tissue, it is possible, though rather improbable, that in the experiment just cited, the thyroid apparatus of the iodotyrosine-fed animals took up the iodine pouring into the organism and elaborated excessive quantities of the hormone, thus inducing metamorphosis. However, the rapid transformation of both thyroidless frog and salamander larvæ when fed or injected with iodized proteins and amino acids renders such an assumption doubtful.

It is interesting to note that Huxley and Hogben metamorphosed *Salamandra* and *Triton* larvæ by rearing them in dilute solutions of inorganic iodine. But in these experiments the thyroid glands of the animals were intact, hence it is impossible to know whether the action of the iodine was direct or through the mediation of the thyroid.

III. Experiments on *Amblystoma punctatum*.

It was considered desirable to test the effects of iodo- and bromtyrosine upon the metamorphosis of *Amblystoma* since it was owing to negative results obtained by administration of inorganic iodine to animals of this group that led Uhlenhuth to assert that iodine has no effect upon salamander transformation.

Eighty young larvæ of *Amblystoma punctatum*, averaging 30 mm. total length were divided into four groups of twenty animals each. The larvæ of three groups were isolated in finger bowls containing 50 c.c of water, one animal to each container. The twenty larvæ remaining were reared in large aquaria and fed quantities of tubifex. The animals in the finger bowls received food only at definite intervals and in very small amounts so that they were in a state of semi-starvation during the course of the experiment.

Twenty larvæ received small amounts of diodotyrosine crystals dissolved in the 50 cc. of water in the finger bowls. Twenty

larvæ were fed equal quantities of dibromtyrosine crystals, whereas the remaining twenty animals received no food of any kind and served as controls. Such a culture was considered necessary in order to note any effects starvation might bring about on the progress of metamorphosis since the animals reared on the tyrosine compounds were given very little food.

It should be pointed out here that the animals in the dibromtyrosine solutions received considerably more bromine than the animals of the iodotyrosine cultures received iodine, despite the fact that equal quantities of the two substances were fed to each larva. This is obvious enough since the atomic weight of iodine is 126.92, while that of bromine is but 79.92. Consequently if equal amounts of the two tyrosine compounds were fed or put into solution in given amounts of water as was the case in the present experiment, the number of bromine atoms per milligram of dibromtyrosine would be nearly double the number of iodine atoms per milligram of diiodotyrosine. In all the experiments recorded in this paper this fact has been ignored and the two tyrosine compounds have been administered in equal amounts as though they were chemically equivalent. The experiment began June 22, 1923. On this date none of the small immature larvæ revealed the slightest indication of metamorphosis. June 28 all animals of the iodotyrosine-fed culture showed marked reduction of the gills—three animals had only the stumps remaining. The tail-fin was undergoing reduction. Sand was placed in each finger bowl in order that the animals might crawl out of the water as metamorphosis progressed; this is a necessary precaution, otherwise the animals will drown over night.

Examination of the larvæ reared in the dibromtyrosine solution showed no change; this was also true of the fed and unfed control cultures.

July 4, twelve days from the beginning of the experiment, all of the diiodotyrosine-fed larvæ had completely transformed and left the water. Their gills and tail-fin had disappeared; the branchial clefts had closed and the larvæ had shed their skins but the pigmentation characteristic of the adults of this species had not appeared. Fig. 1, *A*, is a photograph of four iodotyrosine-fed animals. Compare this with Fig. 1, *B*, which

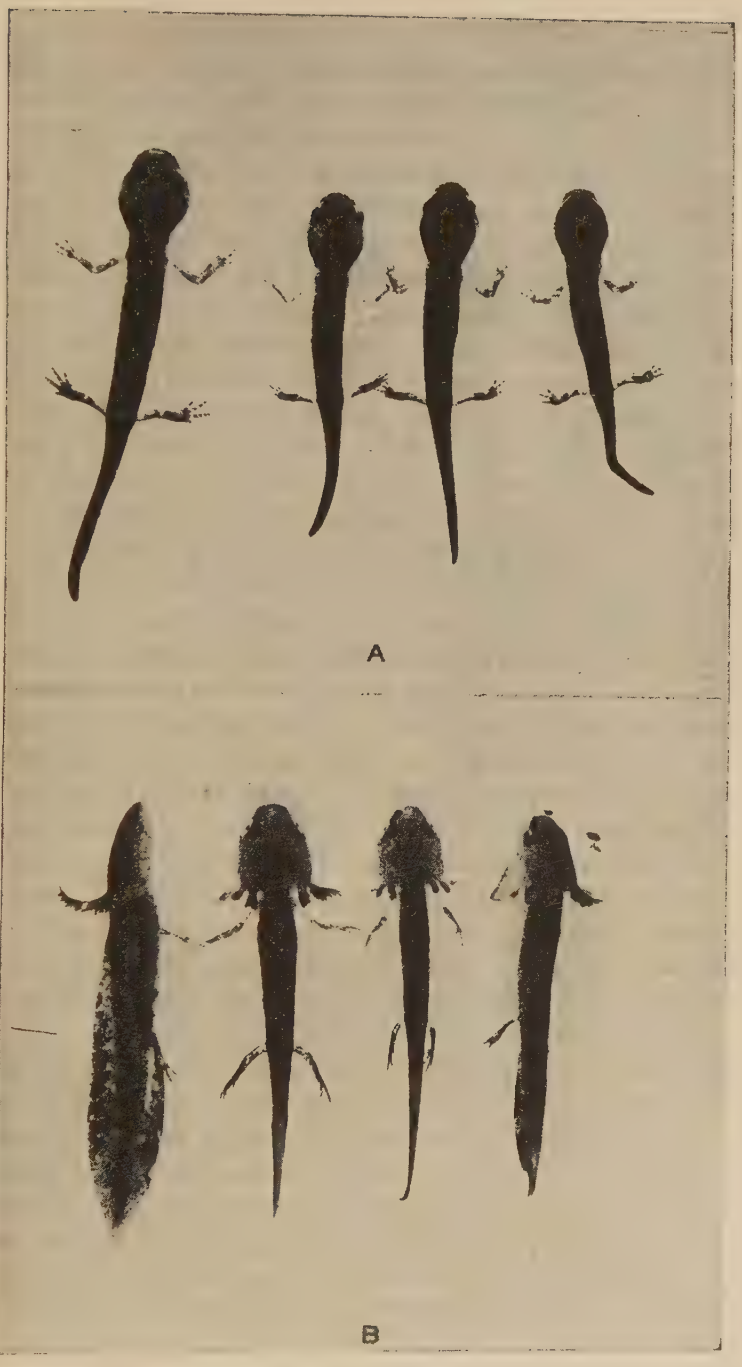

Fig. 1. *A*. Precocious metamorphosis of immature *Amblystoma* larvæ after twelve days immersion in iodotyrosine solution. × 2. *B*. Immature larvæ of *Amblystoma* after twenty-five days immersion in dibromtyrosine solution. × 2.

shows four di-bromtyrosine-fed larvæ. The animals in Fig. 1, *B*, were photographed twenty-five days after the experiment began, hence had been on the dibromtyrosine diet twice as long as the animals of Fig. 1, *A*, had been fed iodotyrosine. The metamorphosed animals were very weak and died within three to five days after metamorphosis. During the twelve days the experiment continued, they were each fed three small worms, the animals of the other cultures (except one) received the same.

The dibromtyrosine culture was continued twenty-six days after transformation of the iodotyrosine culture had occurred, *i.e.*, thirty-eight days from the date of first feeding. At the end of this time eleven animals metamorphosed, the remaining larvæ did not transform. The culture was abandoned July 31. The fed and unfed control groups were also given up at this time.

It is an interesting fact that none of the control animals of either starved or fed cultures metamorphosed during the thirty-eight days of the experiment, whereas eleven larvæ of the dibromtyrosine culture did transform. The experiment indicates that unfed salamander larvæ can be forced to metamorphose if reared in very strong solutions of dibromtyrosine over long periods. The bromine in the tyrosine molecule is thus seen to be not entirely inert as regards metamorphosis though it cannot be compared with iodine. For instance, diiodotyrosine solutions of about one-half the concentration of the dibromtyrosine, metamorphosed salamander larvæ of equal size and developmental stage in a period ranging from seven to twelve days, whereas the much stronger bromtyrosine solutions caused about two-thirds of the larvæ of the culture to transform between the thirtieth and thirty-ninth day.

In an earlier paper ('22) the writer called attention to the fact that the hind legs of thyroidless and pituitaryless tadpoles reared in strong dibromtyrosine solutions grow larger than the limbs of like animals on an algæ diet but that such animals do not metamorphose. Here again the evidence indicates that the bromine ion is not entirely passive since it does stimulate to a slight degree the growth of the tadpole's hind limbs. But the degree of activity of the bromine in the tyrosine molecule is not comparable to that of iodine. The following experiment clearly shows the great difference in activity between the two substances. Three sets of

Fig. 2. *A*. Immature *Rana clamitans* larva metamorphosed by immersion in iodotyrosine solution for fifteen days. *B*. *Rana clamitans* tadpole reared for twenty days in dibromtyrosine solution.

thyroidless and pituitaryless *R. sylvatica* larvæ were used. One set of animals was reared in tyrosine solution, another in iodotyrosine, a third set in dibromtyrosine of twice the strength of the iodotyrosine solution. No food of any kind was given. The iodotyrosine cluture metamorphosed within fifteen days, the animals of tyrosine and dibromtyrosine culture showed no indications of metamorphosis after forty-three days. However, the larvæ reared in the dibromtyrosine solutions had well developed hind legs averaging 7 mm. total length as compared with an average of 3.5 for the animals of the tyrosine culture. Since no food of any kind was given the increase in limb growth was probably due to a slight influence exerted by the bromine ion.

The result of this experiment upon *Amblystoma punctatum* larvæ is identical with that obtained with *Spelerpes*, and shows clearly that it is the iodine in the tyrosine molecule that is responsible for the precocious metamorphosis. The rapidity with which the metamorphic response to iodotyrosine is evoked in salamander larvæ, would seem to indicate that the action of the iodized amino-acid is directly upon the cells and tissues of the organism and not necessarily through the intermediation of the thyroid gland. It will be recalled, that young thyroidless axolotls (*Amblystoma tigrinum*) transform following injections of iodotyrosine and it is probable that thyroidless *Amblystoma punctatum* would react likewise. However, no experiments were made to test this point.

The writer now has under way a series of experiments upon the metamorphosis of thyroidectomized salamander and anuran larvæ in which various iodized proteins and amino acids are fed and injected. The results will be communicated later.

IV. Experiments on *Rana clamitans* Tadpoles.

The larval life of the green frog, *Rana clamitans*, extends over a year (370–400 days according to Wright, '14) and in some individuals is prolonged two years before metamorphosis occurs. This form offers exceptional opportunities for experimentation because of the long duration of larval existence, and is especially valuable for use in investigations on metamorphosis where the feeding method is employed. Tadpoles of all sizes are obtainable at any season of the year and when brought to the laboratory

readily adapt themselves to the changed environment. Animals captured in the autumn generally pass the winter and spring as tadpoles.

September 13, 1922, a large number of larvæ were collected and eighty tadpoles of approximately equal size and developmental stage were selected and divided into four groups of twenty individuals each. One group was fed tyrosine, another ordinary tadpole food such as spirogyra and insect larvæ, the third lot received dibromtyrosine and the fourth group diiodotyrosine. A fifth culture of animals, forty in number, of varying size and developmental stage was kept in large glass aquaria and fed quantities of algæ. Each culture except the fifth was further subdivided into lots of five tadpoles each, and placed in 250 cc. of tap water containing 120 mg. of the tyrosine compounds. Each evening the animals were transferred to large jars containing 10,000 cc. of tap water and plentifully supplied with algæ. This procedure was considered necessary in order to rule out the starvation factor since tadpoles can not exist indefinitely on tyrosine alone.

The animals obtained the tyrosine compounds by two methods: through the alimentary tract, and by absorption through the skin. Most of the tyrosine goes into solution after a short time, hence the larvæ probably obtain most of this substance through the skin. This is certainly the manner in which *Spelerpes* larvæ obtain the tyrosine and its iodized and brominated compounds.

Table I. gives the average measurements of the animals at the beginning and end of the experiment. The figures are averages based upon measurements of fifteen animals of each culture.

TABLE I.

	Algæ-Fed.		Tyrosine.		Dibromtyrosine.		Diiodotyrosine.	
	Total Length, mm.	Hind Legs, mm.	Total Length, mm.	Hind Legs, mm.	Total Length, mm.	Hind Legs, mm.	Total Length, mm.	Hind Legs, mm.
Sept. 13.....	51.2	3.9	52	3.5	52.2	3.8	51.5	3.5
Oct. 8.....	52.2	3.9	52.5	3.8	52.5	3.9	Advanced stages of metamorphosis	

Thirteen days from the beginning of the experiment a marked

difference was observed between the iodotyrosine-fed animals and those of the other cultures. The tyrosine and dibromtyrosine-fed tadpoles showed no changes either in regard to growth or metamorphosis from the algæ-fed controls, whereas the iodotyrosine-fed animals appeared thin and emaciated; the hind legs had increased in length also the skin of the pectoral region where later the fore-legs appear, was undergoing autolysis.

October 3, four of the iodotyrosine-fed tadpoles had fore legs and frog mouths. The hind legs of all of the animals had markedly increased in length, and the tail was undergoing resorption. In several individuals large patches of skin over the region of the fore-limbs was totally destroyed by autolysis. The animals of the remaining cultures showed no change.

October 6, the animals of the iodotyrosine culture were all in advanced stages of metamorphosis and many were dying; only one animal remained alive on October 8. Table I. gives the measurements of the tadpoles of the various cultures at the close of the diiodotyrosine experiment, twenty-three days after the date of first feeding.

The remaining cultures were continued until October 30. The tyrosine and dibromtyrosine-fed tadpoles were then taken off the tyrosine diet and fed algæ. The larvæ were measured November 13 two months after the beginning of the experiment but no indications of metamorphic change was observed. It is quite evident that insofar as the metamorphosis of the green frog is concerned, tyrosine and dibromtyrosine are ineffective, even when administered in large quantities. These results are in agreement with those obtained in similar experiments on thyroidless axolotls and thyroidless and pituitaryless anuran tadpoles (Swingle, '22).

Discussion.

The experiments described in this paper and elsewhere on the administration of iodine, iodized amino-acids and proteins to thyroidless and pituitaryless anuran larvæ and to thyroidless axolotls demonstrate that other forms of iodine than that peculiar to the thyroid hormone (thyroid iodine) possess the power of inducing amphibian transformation. This property of iodine is apparently unique, since so far as known at present it is not shared by other substances, and is inherent in the iodine atom when

organically combined in a certain way. The type of combination is not necessarily that characteristic of thyroid iodine because a large number of iodine compounds have the power to bring on metamorphosis in thyroidless amphibians—even elemental iodine itself. That elemental iodine is utilized within the organism as iodine per se for any purpose seems improbable because if one compares the effect of administering inorganic iodine and various organic preparations to thyroidless tadpoles, it becomes evident that the physiologic activity and metamorphosis-inducing properties of the organic preparations are superior to elemental iodine. Furthemore, the accelerating effect upon metamorphosis of the organic iodine compounds is less than that of the thyroid extract itself. However, the important thing is not the speed with which iodine compounds induce metamorphosis in comparison with the thyroid hormone itself, but the fact that iodine other than thyroid iodine induces the metamorphosis of thyroidless animals. The crux of the problem in regard to amphibian metamorphosis, is to find out just what it is in tyrosine, serumalbumen, serumglobulin, casein, tyramine and probably a host of other amino acids and proteins which when iodine is added increases so remarkably the metamorphosis-inducing powers of this element. Why, for example, does the linking of iodine to the third and fifth carbon atoms of the benzene ring in the tyrosine molecule transform this inert (so far as metamorphosis is concerned) amino acid into a highly active agent. What is it in casein, albumen, globulin or tyrosine that raises so greatly the reactive powers of iodine?

There can be little doubt that elemental iodine when it induces the transformation of thyroidless and pituitaryless tadpoles combines either with the proteins of the algæ fed along with it, or within the body of the tadpole after absorption through the skin or alimentary tract, but it doesn't combine with anything produced by thyroid tissue because there was none present nor had there ever been any present in the case of the thyroidless anurans.

Hirschler ('22) precociously metamorphosed tadpoles by inserting small pieces of elemental iodine into the body of the larvæ. However, since the animals possessed intact thyroid glands their activity can not be ruled out in this experiment,

because the metamorphosis may have been due to increased activity of the thyroid due to increased iodine supply. On the other hand the absorbed iodine may have combined with other than thyroid proteins thus inducing metamorphosis. Either or both of these possibilities may have been realized in Hirschler's experiment, consequently work involving iodine administration should be performed only on thyroidectomized larvæ since only this type of experiment will shed any light upon the rôle of iodine in amphibian metamorphosis. This is especially true of neotenous forms like axolotl; no work on the rôle of iodine in the metamorphosis of this form should be regarded as conclusive unless performed upon animals from which all trace of thryoid tissue has been removed.

Thyroid conditions in axolotl are very peculiar (Swingle, '22) and vitiate the results of experiments done on animals with intact glands. The New Mexican strain of axolotl has perfectly developed thyroids, the vesicles filled to capacity with the physiologically active hormone yet the secretion is apparently unable to escape into the blood stream in sufficient quantities to transform the animal. This is demonstrated by heteroplastic thyroid transplants. An axolotl thyroid is sufficient to metamorphose thirteen normal, thyroidless and pituitaryless anuran tadpoles when grafted, but left intact within the axolot's body is incapable of initiating metamorphosis. [1] It is obvious that in this form we are dealing with a thyroid mechanism which selects, stores and transforms the iodine of the animal's food and water into the thyroid hormone but fails to release the elaborated product. Consequently, if axolotls are fed elemental iodine they do not metamorphose, and for exactly the same reason, they fail to transform under the ordinary dietary regime—the iodine is picked up by the gland and synthesized but not released. It is probable that feeding organic iodine preparations would give similar results. On the other hand it is erroneous to conclude from such an experiment that iodine has no influence on axolotl metamorphosis. Our experiments on both thyroidectomized and partially thyroidectomized axolotls show quite clearly that iodized amino acids and proteins promptly metamorphose these

[1] This experiment was performed by Mr. Karl Mason, of this laboratory, and reported at the Boston meeting of the American Society of Zoölogists, 1922.

animals, and that the amino-acid and protein without the iodine in the molecule are inert.

Hirschler ('22) metamorphosed axolotls (the European strain which rarely spontaneously transforms) by implanting iodoform paste within the body cavity. However, in this experiment the glands were intact so it may be that the iodoform merely served to stimulate the thyroid mechanism to release its stored hormone thus inducing metamorphosis. It will be recalled that Kaufman ('18) metamorphosed axolotl by injections of salicylic acid. So far as is known salicylic acid has no influence on metamorphosis, the effect of injecting the substance into axolotl was to stimulate the secretory activity of the thyroid apparatus in some way. Anyone not familiar with thyroid conditions in axolotl might conclude that salicylic acid per se was the metamorphosis-inducing agent, whereas probably nothing could be farther from the real facts of the case.

The New Mexican strain of axolotl if removed from its native habitat to New Haven soon undergoes spontaneous metamorphosis. Why? Certainly not because the railroad journey exerts any mysterious metamorphosis-inducing power, but probably because the changed food, water, the jolting and confinement incident to the trip acted as a stimulating agent thus releasing the thyroid hormone from the gland vesicles thereby causing transformation.

Where the thyroid apparatus is left intact the fate of the substances fed or injected into an amphibian larva is problematical. If an effect upon metamorphosis is produced it is impossible to determine whether the effect is due directly to the substance itself, or indirectly through intermediation of the thyroid unless the work is checked by repeating it upon thyroidless forms. An excellent illustration of this statement has been furnished by Allen ('20). Allen observed that transplantation of the anterior lobe of the pituitary into hypophysectomized tadpoles (but possessing a rudimentary and functionless thyroid apparatus) metamorphosed the animals, whereas transplantation of the pituitary gland into thyroidectomized larvæ had no such effect. The pituitary secretion had no direct influence upon metamorphosis but acted indirectly by stimulating the rudimentary thyroid into functional activity. Any one working with

normal tadpoles with intact thyroids uncontrolled by thyroidless animals might well have concluded from the results of such an experiment that pituitary tissue exerts a direct stimulus to metamorphosis and is thus equivalent to the thyroid.

Recently ('22) Romeis claims to have isolated an absolutely iodine-free substance from the thyroid gland which when administered to frog tadpoles exhibits all of the physiologic effects of thyroid gland tissue. The writer is skeptical of the validity of this claim and for several reasons: (1) All active substances so far isolated from the thyroid contain large amounts of iodine. Thyroxin the active principle contains sixty-five per cent. of iodine as an integral part of the molecule; and it is known that thyreoglobulin containing no iodine is physiologically inert; (2) Experiments on tadpoles have shown (Rogoff, '18–19) that blood coming from hyperplastic thyroid glands with extremely low iodine content fails to induce tadpole metamorphosis; (3) To date the only substances known to metamorphose thyroidless tadpoles are thyroid or iodine in some form—presumably in the last analysis organically combined. While writing this paper the writer came across a second communication from Romeis which practically amounts to a retraction of his earlier claims. He found that iodothyrine and iodothyroglobulin will induce the metamorphosis of tadpoles in dilutions of one in a million; thyroxin produced the same effects on growth and metamorphosis in dilutions of one in ten million consequently says Romeis: These figures suggest that effects (on tadpoles) from so-called iodine-free materials from the thyroid may have been contaminated with minute amounts of thyroxin.

There can be little doubt that this is the real explanation of the results obtained by this investigator with so-called iodine-free substances from the thyroid.

There seems to exist a fundamental difference between mammals and amphibians in regard to their physiologic response to thyroid and iodine administration. This difference is not sufficiently understood by those who attempt to compare these two verebrate groups. The following experiment of Kendall ('19) is an excellent illustration of the point: He found that injections of pure thyroxin into mammals is followed by a very definite and marked physiologic response. But when the hydrogen of the

imino group in the thyroxin is replaced with acetyl, the substance loses its physiologic activity and there follows no demonstrable effect upon the metabolic rate. This emphasized the importance of the imino group in thyroxin (in so far as the metabolic effect upon mammals is concerned) and minimizes the importance of the iodine in the molecule. Bearing in mind the effect of iodine administration upon tadpole metamorphosis, Kendall was led to try the acetyl derivative of thyroxin on tadpoles, for if metamorphosis depends only upon the increase in the basal metabolic rate of the larvæ then thyroxin should increase the rate of metamorphosis but the acetyl derivative involving the imino group should not. If, however, iodine alone is concerned in accelerating metamorphosis, then both thyroxin and the derivative should affect the transformation. Kendall found that both thyroxin and the acetyl derivative would induce a rapid metamorphosis of the bull frog tadpole. Kendall's conclusion was that thyroxin appears to have two separate and distinct functions: the effect upon the metabolic rate which is brought about by the CO-NH groups within the molecule; and the physiological changes involved in the metamorphosis of the tadpole due to the iodine contained in the molecule. Our own experiments have demonstrated that this action of iodine is not specific to thyroxin, but can be obtained in thyroidless amphibians (though the effects are not so rapid, and the amounts administered must be larger) by a large number of other iodine compounds and by administration of elemental iodine itself.

Kendall's experiment sheds considerable light on the reason for the conflicting results obtained by investigators working with iodine, iodized proteins and amino acids on mammals, and the students of amphibian metamorphosis. In mammals the criterion employed for testing the phsiologic action of iodine and thyroid upon the organism is the effect upon metabolism as indicated by changes in the nitrogen excretion, CO_2 elimination and oxygen consumption; in amphibians the criterion has been the rate and degree of the degenerative and regenerative processes incident to metamorphosis. However, it is becoming clear that the two types of physiologic response are not in the same class and hence not to be compared because they owe their origin to different causes. The unique effects of thyroid or thyroxin upon the meta-

bolic rate is due to the specific chemical structure of the thyroxin molecule particularly the CO–NH group, whereas metamorphic response of amphibians is dependent upon a peculiar property of iodine in certain types of combination, although not necessarily that characteristic of thyroid iodine, *e.g.*, iodized amino acids and proteins. Further evidence of the difference as to cause between metabolic changes in mammals and amphibian metamorphosis is furnished by the acetonitrile test where iodized substances shown to be specific in accelerating metamorphosis completely fail to simulate the thyroid function in protecting mice against the lethal effects of actonitrile.

Hunt and Seidell ('09) made the interesting observation that feeding thyroid tissue to white mice greatly increases the resistance of these animals to lethal doses of acetonitrile, and that the efficiency of the gland seems dependent upon its iodine content. They concluded that the increased resistance of the mice to the poison was due to the changed metabolism of the animals following thyroid feeding, the metabolic change preventing the acetonitrile from breaking down into its posionous product hydrocyanic acid. This assumption was based upon the fact that thyroid feeding does not raise the resistance of mice to lethal doses of hydrocyanic acid itself.

Koch ('13) and Miura ('22) found that iodized amino acids such as diiodotyrosine iodotryptophan and tetra-iodohistidine when administered to mice fails to increase their resistance to acetonitrile.

Strouse and Voegtlin ('09–'10) failed to observe any thyroid-like effect on the nitrogen metabolism or on the blood pressure of normal dogs, nor was there any favorable effect on the condition of myxedematous and cretinous mammals following administration of iodized amino acid. Other investigators have tried in vain to obtain thyroid effects on mammals (metabolic changes) by the use of various iodized substances; tri-iodo-imidazol and, iodophenylalanie have also proven ineffective.

If, however, we bear in mind the results of Kendall's experiment, it becomes clear why iodized amino acids, *e.g.*, iodotyrosine, give negative results when administered to myxydematous and cretinous mammals, and positive results when fed to thyroidless amphibian larvæ. In the latter group the metamorphic response

is due to the iodine in the molecule, in the former group the metabolic response depends upon something else, *i.e.*, the CO–NH group within the thyroxin molecule. This brings us to the consideration of another point, *i.e.*, the possibility of substituting other halogens for iodine and obtaining the same effects upon metamorphosis.

Kendall ('18) as a result of his investigations of the unique effects of thyroxin upon the metabolic rate of mammals, was led to conclude that insofar as the physiologic effect upon mammals is concerned, possibly other halogens could be substituted for the iodine of the thyroxin molecule without greatly changing the physiological properties of the thyroxin. His conclusion follows: "In regard to the relation of iodine to the activity of thyroxin, the presence of iodine in the compound must exert some influence, and it seems not improbable that the presence of iodine renders the active groups more reactive. In the absence of iodine it would take a greater working pressure to bring about its reaction. The substitution of iodine by hydrogen or chlorine or bromine would undoubtedly be followed by an alteration in the degree of reactivity of the substance but its gross chemical nature and properites would not be altered thereby."

Whether or not other halogens can or cannot be substituted for the iodine of the thyroxin molecule and this substance still retain its physiological activity in mammals is an open question, at any rate there are no experimental data tending to answer the question one way or the other except possibly the work of Ostwald and von Cyon who observed that thyreoglobulin containing no iodine was physiologically inert, whereas this substance gives all the physiological effects of thyroid tissue when iodine is present. However, this may be, it is clear that insofar as amphibian metamorphosis is concerned other halogens such as bromine can not be substituted for iodine. The experiments upon thyroidless axolotls and anuran larvæ where 3-5 dibromtyrosine was employed, demonstrates the futility of endeavoring to substitute bromine for iodine with hope of affecting metamorphosis. It is merely quibbling to say that bromine would probably be just as effective as iodine providing the organism possessed a mechanism for utilizing this halogen in the way the thyroid utilizes iodine to elaborate its hormone, because in our experiments the animals

fed iodotyrosine and iodoserumglobulin had no vestige of thyroid tissue present, consequently had no mechanism for iodine utilization.

No one has ever shown that in the absence of thyroid glands, other tissues of the organisms have the power of functioning vicariously for the thyroid, and synthesizing its active hormone. If this possibility were true then why is it that mammals with atrophied or degenerate thyroids are quite unable to utilize iodine or iodized proteins and amino-acids. If other tissues of vertebrates besides the thyroid glands possess the power to manufacture the thyroid hormone it is strange that this power should be present in amphibia yet lacking in mammals. The truth of the matter is that amphibian metamorphosis depends upon a peculiar property inherent in the iodine atom when combined in certain ways.

Some investigators have claimed that the pituitary can function vicariously for the thyroid when the latter is absent, hence it might be said that in thyroidless amphibians the pituitary gland may synthesize the iodine into the chemical complex characteristic of the thyroid hormone. Aside from the total lack of evidence that the pituitary can function vicariously for the thyroid in thyroidless forms, the experiments of Allen ('19) are of interest in this connection. Allen extirpated both the thyroid and the pituitary gland of frog embryos and later fed the tadpoles with starch iodide. The animals underwent a precocious and nearly complete metamorphosis before death ensued, clearly demonstrating that iodine is as effective in inducing transformation in tadpoles lacking both thyroid and pituitary as in larvæ with only the thyroid missing.

Summary of Conclusions

1. Thyroidectomized, partially thyroidectomized, and normal axolotls are readily metamorphosed by intraperitoneal injections of iodotyrosine and iodoserumglobulin and iodocasein.

2. Thyroidectomized, partially thyroidectomized and normal axolotls do not metamorphose when injected with large quantities of pure tyrosine, 3-5 dibromtyrosine (two atoms of bromine in the molecule) and non-iodized serumglobulin.

3. Larval *Spelerpes bilineatus* readily metamorphoses in strong solutions of 3-5 diiodotyrosine, but do not transform in equivalent concentrations of tyrosine and 3-5 dibromtyrosine even when kept in such solutions over comparatively long periods.

4. *Rana clamitans* tadpoles with from six to eight months of larval life remaining (*i.e.*, passing the winter as tadpoles) were metamorphosed within twenty days by rearing the animals in strong concentrations of 3-5 diiodotyrosine and feeding them with this substance. Control larvæ of similar age and developmental stage reared in equivalent solutions of 3-5 dibromtyrosine and fed quantities of the compound failed to transform.

5. The experiments are clean cut and admit of but one interpretation; it is the iodine in the amino acid and protein molecule that is responsible for amphibian metamorphosis. Why the linking of iodine to the third and fifth carbon atoms of the benzene ring of the tyrosine molecule should so greatly increase the reactive powers of the iodine is unknown.

6. Iodine other than thyroid iodine is effective in inducing the metamorphosis of thyroidless urodele and anuran larvæ. There is no evidence that any other tissue of the vertebrate organism has the power to function vicariously for the thyroid in the latter's absence.

7. Bromine has little or no influence upon amphibian metamorphosis and can not be substituted for iodine. The substitution of two bromine atoms for two hydrogen atoms (the third and fifth) of the tyrosine molecule fails to change this substance into a metamorphosis-inducing agent.

8. The physiologic responses (metabolic changes) of mammals to thyroid administration are due to the CO–NH group within the thyroxin molecule; amphibian metamorphosis to the iodine in the molecule. Consequently thyroidless animals of these two vertebrate groups are hardly comparable in regard to their response to iodine. Only thyroid iodine containing the CO–NH group is effective in thyroidless mammals whereas iodine other than thyroid iodine will metamorphose thyroidless amphibian larvæ.

9. Data are presented showing why experiments on amphibian larvæ devised to test out the effect upon metamorphosis by feeding or injecting various substances are unsound unless per-

formed upon thyroidless animals or at any rate controlled by identical experiments upon thyroidectomized individuals.

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